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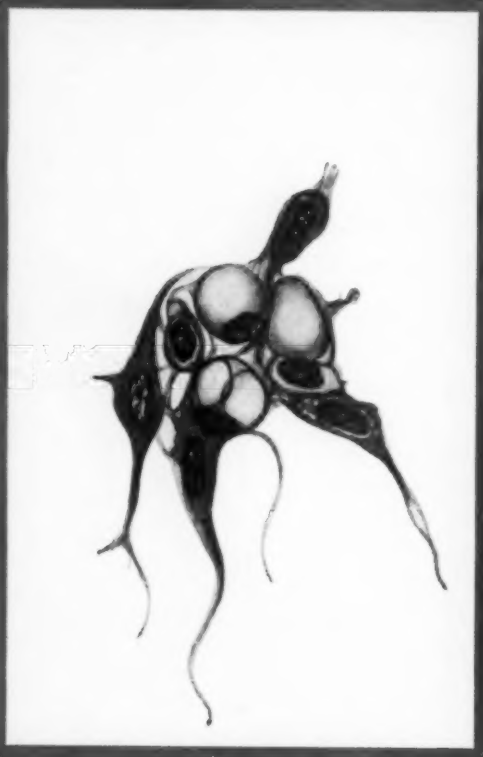

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Experimental Fibrinoid Lesions in Rabbits

Production by Cross Transfusion and Isolated Renal Perfusion

CHARLES N. GAMBLE, M.D.
and
JOEL G. BRUNSON, M.D., Minneapolis

Previous papers have defined the morphologic changes occurring in the tissues of rabbits after the intravenous administration of a single injection of meningococcal endotoxin,[1] two appropriately spaced injections of endotoxin,[2] and the injection of endotoxin in combination with or after the intravenous administration of certain high molecular weight acidic polymers.[3] The most striking feature of the changes following these procedures is the presence of occlusive masses of a dense homogeneous eosinophilic material in the glomerular capillaries, splenic sinusoids, hepatic veins, and pulmonary arteries, as well as within the cardiac valves and beneath the endothelium of the coronary arteries. In association with its deposition varying degrees of congestion, hemorrhage, and necrosis are observed in the involved organs. This material has the tinctorial features described by Altshuler and Angevine [4] as being typical for fibrinoid.

The widespread distribution of this material within the cardiovascular system and the inhibition of its formation by heparin * suggest that either some alteration in the blood accounts for its formation or that it is formed in other tissues and then enters the blood stream. The present experiments were designed to test this hypothesis to see if typical lesions could be produced by the passive transfer of fibrinoid to recipient animals and organs.

MATERIAL

A total of 145 hybrid albino rabbits of both sexes, each weighing 1 to 1.5 kg., were used. They were fed Purina rabbit pellets and had free access to water. Following death or killing of the animals, the tissues were fixed in 10% neutral formalin. Sections were routinely taken from the kidneys, spleen, liver, heart, lungs, pancreas, and adrenal glands. Hematoxylin and eosin were used as a routine stain, and many additional sections were stained by the periodic acid-Schiff method. Selected sections from the kidneys were stained with phosphotungstic acid hematoxylin, toluidine blue, crystal violet, and Van Gieson's stain.

Meningococcal endotoxin was used throughout the experiments. Details of its preparation have been reported elsewhere.[7] It was diluted 1 : 80 or 1 : 1000 with sterile, pyrogen-free, isotonic saline solution and was injected into the marginal ear vein of the animals in a volume of 2 cc.

Sodium polyanethol sulfonate (Liquoid) was obtained from Hoffmann-La Roche, Inc. This high molecular weight acidic polymer possesses anticoagulant properties somewhat similar to heparin,

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From the Department of Pathology, University of Minnesota Medical School.

These studies were supported by grants from the Minnesota Heart Association, the American Heart Association, and the Youngstown Area Heart Association.

Part of this work was done during the tenure of a Lederle Research Scholarship (Dr. Gamble). This paper won the 1955 Minnesota Borden Award for student research, and also won one of five equal awards given by the General Research Committee of the American Association of Clinical Pathologists.

* References 5 and 6.

and part of its activity appears to be related to a combination with fibrinogen.[8] When it is administered intravenously in conjunction with an injection of meningococcal endotoxin, a majority of animals develop lesions identical with those which follow two appropriately spaced intravenous injections of endotoxin. This material was dissolved in isotonic saline solution, passed through a Seitz filter, and injected in doses of 10 mg. into the marginal ear vein.

Heparin sodium (Parke, Davis & Company) was used in certain of the experiments and was injected in doses of 5 mg. into the marginal ear vein.

The polyethylene tubing was obtained in two sizes to facilitate easy connection during transfusion and perfusion. The pump used in perfusing isolated kidneys was a Sterling automatic pipette manufactured by Ivan Sorvell Inc., New York, and it proved very satisfactory in delivering the small quantities of blood desired.

METHODS

Carotid-Jugular Cross Transfusion Experiments.—One group of donor animals was prepared by the intravenous injection of 2 cc. of 1:80 meningococcal toxin. After an interval of 18 to 24 hours they were given a second, or shocking, injection of an equal volume of the same dilution of endotoxin. Another group was given a simultaneous intravenous injection of 2 cc. of 1:1000 meningococcal toxin and 10 mg. of sodium polyanethol sulfonate. Cross circulation experiments were performed two to four hours after the second injection of endotoxin or the injection of endotoxin and sodium polyanethol sulfonate. Control donor animals were given only a preparatory injection of toxin or were unprepared. The recipient animals, with the exception of one group given a single intravenous injection of 2 cc. of 1:80 toxin were unprepared.

Preparation for cross transfusion consisted of exposing an external jugular vein and internal carotid artery of each animal under local procaine anesthesia. After placement of ligatures about these vessels, both donor and recipient animals were given 5 mg. of heparin intravenously to prevent clotting within the catheters. After this procedure the vessels were ligated and cannulated with polyethylene tubing. The carotid catheter of the donor animal was inserted into the larger jugular catheter of the recipient, and the recipient carotid catheter was connected to the donor jugular catheter in a similar fashion. The catheters were then opened with immediate transit of blood, and cross transfusion was allowed to continue for periods of 20 minutes to 1 hour. After transfusion the animals died or were killed at periods of time varying from 2 to 24 hours. With the exception of 10 animals from the entire group of 85, all animals survived for 12 to 24 hours.

An additional small group of animals was used to determine if the shocking dosage of toxin administered to prepared donors remained free in the circulation and was able to exert its direct effect in the recipient animal in a manner similar to a single intravenous injection of toxin. For this purpose one group of animals was prepared by the usual method of intravenous injection of 2 cc. of 1:80 toxin, while another group was unprepared. At 24 hours later one of the prepared animals was given an intravenous injection of 0.32 cc. of undiluted toxin, so that the estimated final blood dilution was 1:80 toxin per cubic centimeter. Two hours after this procedure blood was drawn from the heart of the shocked animal and 2 cc. of this blood was injected intravenously into the prepared and unprepared animals, which were then allowed to survive for twenty-four hours.

Isolated Renal Perfusion Experiments.—One group of donor animals was prepared and shocked in the usual manner with 2 cc. of 1:80 meningococcal toxin. They were then allowed to "react" for average periods of three to eight hours until perfusion was begun. Another group of donor animals was given a simultaneous intravenous injection of 2 cc. of 1:1000 meningococcal toxin and 10 mg. of sodium polyanethol sulfonate. An average interval of 10 minutes to 6 hours was allowed to occur in this group before perfusion was begun. A small group of control animals was unprepared. Recipient animals, which were to furnish "recipient kidneys" for the donors given two injections of toxin, were prepared with a single intravenous injection of 2 cc. of 1:80 toxin 18 hours prior to perfusion. The other recipient animals used in the toxin-sodium-polyanethol-sulfonate and control experiments were unprepared.

The donor animals were prepared for perfusion in a manner identical with that used in the carotid-jugular cross transfusion experiments. The animals which were given toxin or which were unprepared were given 5 mg. of heparin intravenously immediately prior to cannulation of the neck vessels and perfusion. The animals given toxin plus sodium polyanethol sulfonate were not given heparin because of the effective anticoagulant activity of sodium polyanethol sulfonate.

Isolation of the kidneys was carried out by exposing the vessels in the region of the left kidney. The inferior vena cava and aorta were isolated above and below the kidney. Separate ligatures were placed about the aorta and inferior vena cava above the left renal artery and vein and below the right renal artery and vein. Ligatures were then placed about the aorta and inferior vena cava below the kidney to make cannulation possible. This procedure completely excluded the right kidney and other organs except the left adrenal gland from the perfusion circuit. Just prior to exclusion of the left

kidney from the animal's circulatory system, 5 mg. of heparin sodium was injected into the marginal ear vein of all recipient animals. After this procedure the aorta and inferior vena cava above the kidney were ligated, and these vessels below the kidney were separately cannulated with polyethylene catheters.

In experiments in which a "prepared" kidney was perfused with the blood of an animal which had previously been given two intravenous injections of toxin, the aortic cannula was connected to the internal carotid artery cannula of the donor animal and the inferior vena cava cannula was connected to that of the external jugular vein of the donor. The cannulae were opened and the blood pressure of the donor animal utilized to effect transit of blood through the perfusion circuit. The isolated kidney slowly became pink in blotchy areas as fresh blood was forced into it, until at 15 to 20 minutes after the start of the procedure the kidney was a normal reddish-pink color. In experiments in which toxin-sodium-polyanethol-sulfonate or unprepared donors were used, the pump was utilized in an attempt to effect more rapid and complete perfusion. It was interposed in the arterial side of the circuit and calibrated to deliver between 15 to 20 cc. per minute. In these experiments almost immediate return of the isolated kidney to normal appearance was observed.

A variable number of donors were used to perfuse one kidney. This was done in the attempt to insure the use of "positive-reacting" donor animals. The duration of perfusion varied between one to five hours. Even at the upper limits of this range the kidney was observed to remain functional, judging by the formation of urine from the catheterized ureter, although studies on the electrolyte content of this urine were not made.

RESULTS

Carotid-Jugular Cross Transfusion.—The results of these experiments are summarized in Table 1.

Initial experiments were performed with the use of recipient animals prepared by the intravenous administration of 2 cc. of 1:80 toxin 18 hours prior to transfusion. Because of the previous observation [1] that a single intravenous injection of toxin resulted in considerable vascular damage, it was believed that preparation might be necessary for the deposition of fibrinoid in the highly vascular areas in which it is typically found. Tissues from the recipient animals in this group exhibited lesions which were morphologically identical with those occurring after the ad-

TABLE 1.—*Incidence of Fibrinoid Lesions Following Carotid-Jugular Cross Transfusion*

Donor Animals										Recipient Animals								
					Presence of Fibrinoid								Presence of Fibrinoid					
No. of Animals	Prep.	Shock	Average Interval After Shock	Kidneys	Spleen	Heart	Liver	Lung	No. of Animals	Method	Average Duration of Transfusion	Kidneys	Spleen	Heart	Liver	Lung
10	Toxin 1:80	Toxin 1:80	2 hr.	5	8	1	1	6	10	Toxin 1:80	30 min.*	2	6	3	4	3
10	Toxin 1:80	Toxin 1:80	4 hr.	7	9	6	7	4	10	No toxin	1 hr.	0	4	6	2	2
8	Toxin 1:1000 + 10 mg. sodium polyanethol sulfonate	.....	1 hr.	6	7	6	2	7	8	No toxin	50 min.	0	3	5	3	4
5	Toxin 1:80	.....	12 hr. postprep.	1	3	2	0	1	5	No toxin	20 min.	0	0	1	0	0
5	No toxin	.....	.....	0	0	2	0	0	5	No toxin	30 min.	0	0	2	0	0

* Interval from preparation of recipient to transfusion 18 hr.
All animals with the exception of the toxin-sodium-polyanethol-sulfonate donors received 5 mg. of heparin prior to transfusion.

ministration of toxin or toxin and sodium polyanethol sulfonate. The renal lesions that occurred in 20% of the animals, however, were not "fully developed" and were noted only after microscopic study revealed the typical homogeneous, eosinophilic material within the glomerular capillaries. Splenic lesions, consisting of accumulations of identical material within the splenic sinusoids, occurred in 60% of the recipient animals. Its occurrence was occasionally associated with splenic congestion and necrosis. Myocardial alterations were present in all recipient animals and consisted of areas of hemorrhage, necrosis, cellular infiltration, and occasional calcification of the muscle fibers. Intravalvular hemorrhages were also noted consistently. Cardiac fibrinoid was observed within the substance of the valves (Fig. 1) and beneath the endothelium of the intramural coronary arteries in 30% of the animals. Focal hepatic necrosis (Fig. 2) and thrombi within the lumina of the efferent venules of the liver were noted in 40%, while thrombi in the small pulmonary arteries (Fig. 3) were seen in 30%, of the transfused animals. The material found in all of these lesions was tinctorially identical with that described previously and designated as fibrinoid.†

With the exception of the low incidence of renal lesions, these results are comparable to those produced in animals given two intravenous injections of toxin.[2] However, there was no way to ascertain definitely whether these lesions resulted because of transfer of fibrinoid, or if the changes that were observed followed the preparatory injection of toxin. For this reason unprepared recipient animals were transfused with the blood of prepared-shocked donors. None of the recipient animals of this group were observed to have renal lesions, but splenic lesions were noted in 40%, cardiac fibrinoid in 60%, and hepatic and pulmonary fibrinoid in 30%, of these animals.

The possibility was then suggested that toxin administered to a prepared donor might remain unchanged and freely circulating in the blood stream for a period of time sufficiently long so that transfusion merely resulted in a situation comparable to that of administering a single intravenous injection of toxin. In the attempt to investigate this possibility, a small group of prepared and unprepared animals was given an injection of 2 cc. of donor blood estimated to contain a dilution of 1 : 80 toxin per cubic centimeter. The results of this procedure are summarized in Table 2. In the prepared recipient group, 14% of the kidneys contained glomerular fibrinoid, whereas 43% of the spleens, 29% of the lungs, and 14% of the livers contained this material. Cardiac fibrinoid was not observed. In the unprepared recipient group fibrinoid was not observed in any of the tissues examined.

In unprepared recipient animals transfused with the blood of donors prepared by the simultaneous intravenous injection of 2 cc. of 1 : 1000 toxin and 10 mg. of sodium polyanethol sulfonate, no renal lesions were observed. Of these animals, 38% developed splenic and hepatic lesions, whereas 63%

TABLE 2.—*Injection of Donor Blood into Recipient Animals*

Donor Animals		Recipient Animals*							
					Presence of Fibrinoid				
Method	Interval After Shock	Method	No. of Animals	Interval After Prep.	Kidneys	Spleen	Heart	Liver	Lung
0.32 cc. of undiluted toxin i.v. 24 hr. after 1:80 endotoxin intravenously	2 hr.	2 cc. 1:80 toxin i.v. 24 hr. prior to 2 cc. of donor blood i.v.	7	26 hr.	1	3	0	1	2
		No preparation; 2 cc. of donor blood i.v.	7	.....	0	0	0	0	0

* Recipients survived 24 hr. after injection of donor blood.

† References 1, 2, 3, and 6.

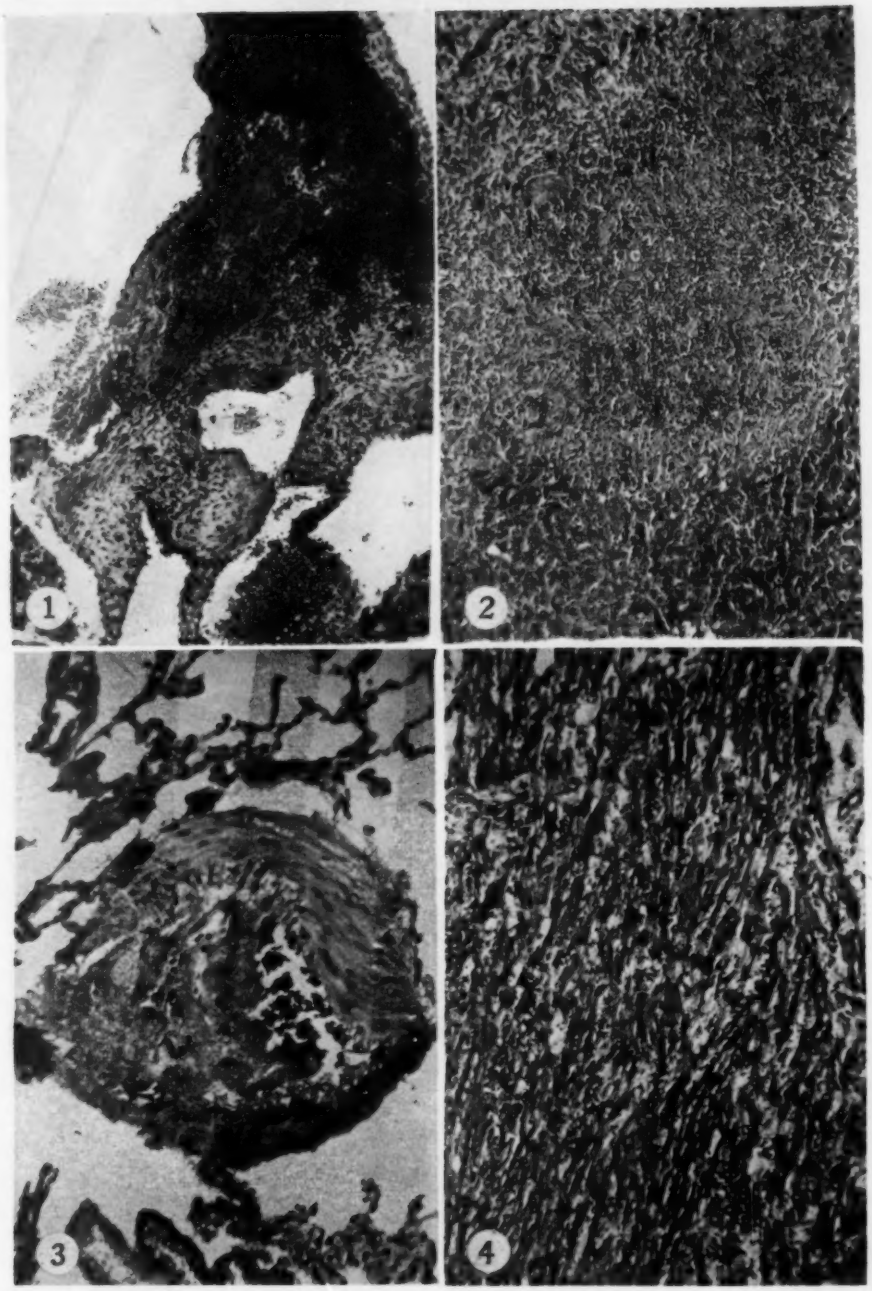

Fig. 1.—Section from the mitral valve of an animal transfused with the blood of a donor animal given two intravenous injections of meningococcal toxin. Note the accumulations of fibrinoid along the free margin and within the substance of the valve, as well as extensive hemorrhage and increased cellularity. Hematoxylin and eosin; × 100.

Fig. 2.—Section from the liver of an animal transfused with the blood of a donor animal given two intravenous injections of meningococcal toxin. Accumulations of fibrinoid are present within an area of focal necrosis. Hematoxylin and eosin; × 100.

Fig. 3.—Pulmonary artery from an animal transfused with the blood of a donor animal given two intravenous injections of meningococcal toxin. A fibrinoid thrombus occludes the vessel lumen. Hematoxylin and eosin; × 175.

Fig. 4.—Section from the myocardium of an animal transfused with the blood of an untreated donor animal. Note fragmentation and necrosis of the muscle fibers and the interstitial accumulation of mononuclear cells. Hematoxylin and eosin; × 100.

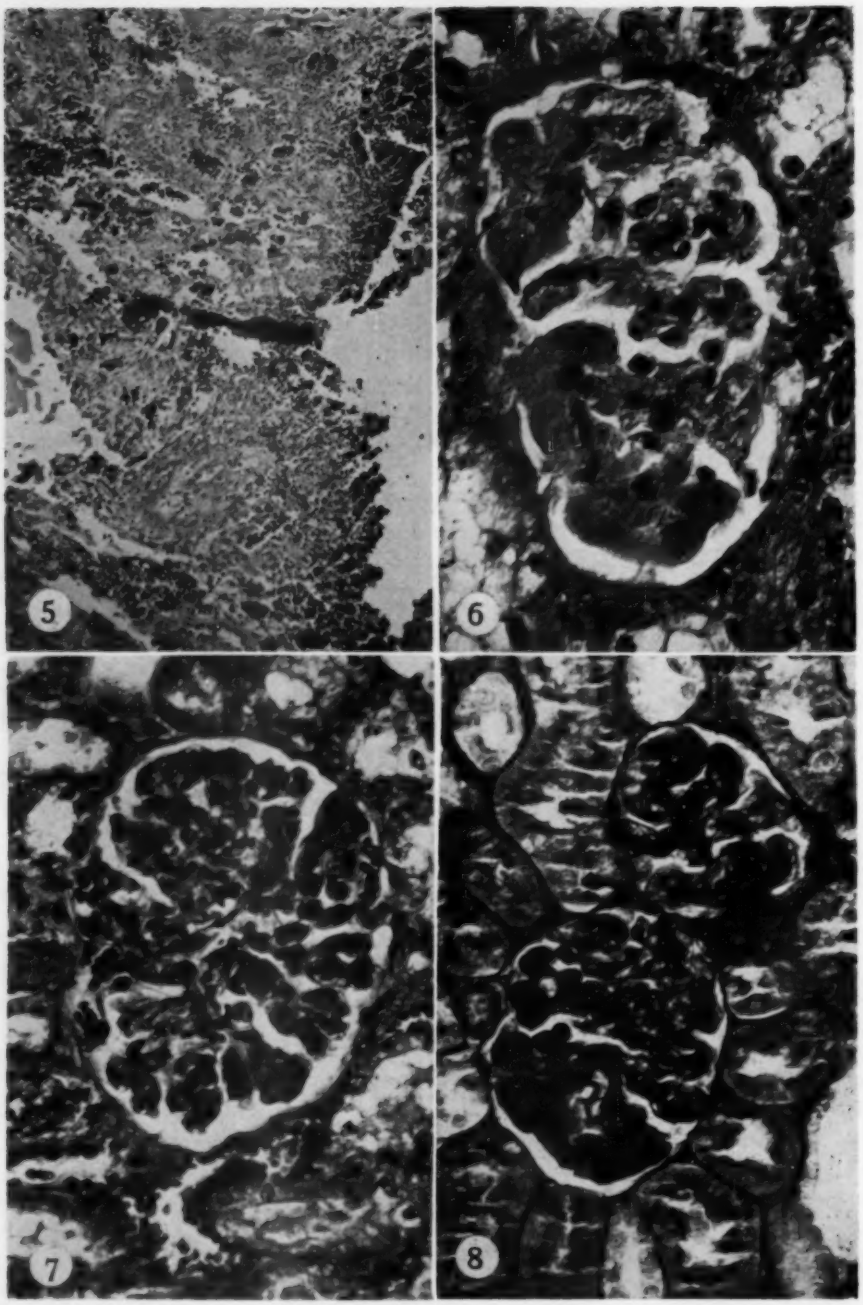

Fig. 5.—Section from the base of the mitral valve of an animal transfused with the blood of an untreated donor animal. There is extensive disruption of the valve with deposition of fibrinoid and areas of focal hemorrhage within its substance. Hematoxylin and eosin; × 100.

Fig. 6.—Glomerulus from a kidney perfused with the blood of an animal given an intravenous injection of meningococcal toxin and sodium polyanethol sulfonate. Fibrinoid fills the lumina of the majority of the capillary loops. Hematoxylin and eosin; × 450.

Fig. 7.—Glomerulus from a kidney perfused with the blood of animal given an intravenous injection of meningococcal toxin and sodium polyanethol sulfonate. Fibrinoid is present within the lumina of the capillary loops and afferent arteriole and is applied against the basement membrane of some of the vessels, producing a "wire-loop" appearance. Hematoxylin and eosin; × 450.

Fig. 8.—Glomeruli from a kidney perfused with the blood of two animals given intravenous injections of meningococcal toxin and sodium polyanethol sulfonate. Periodic acid-Schiff stain. Note the intensely black areas which represent Schiff-positive fibrinoid material within the glomerular capillaries. × 400.

TABLE 3.—*Isolated Renal Perfusion*

Donor Animals (Prepared and Shocked with 2 Cc. 1:80 Toxin)								Recipient Kidney* (Prepared with 2 Cc. 1:80 Toxin)		
									Glomerular Fibrinoid	
No. of Animals	Average Interval After Shock	Average Survival After Shock	Presence of Fibrinoid: Kidney	Spleen	Heart	Liver	Lung	Duration of Perfusion	Perfused Kidney	Non-perfused Kidney
1	3 hr.	9 hr.	0	1	0	0	1	5 hr.	—	—
2	6 hr.	18 hr.	1	2	0	0	1	2½ hr.	—	—
7	8 hr.	20 hr.	2	5	3	3	3	4½ hr.	—	—
5	4½ hr.	5 hr.	2	5	0	4	5	3½ hr.	—	—
6	4 hr.	8 hr.	5	6	1	4	6	4½ hr.	—	—

* Average interval after preparation 24 hr.
Each animal received 5 mg. of heparin prior to perfusion.

TABLE 4.—*Isolated Renal Perfusion*

Donor Animals (2 Cc. 1:1000 Toxin Plus 10 Mg. Sodium Polyanethol Sulfonate)								Recipient Kidney* (No Previous Preparation)		
									Glomerular Fibrinoid	
No. of Animals	Average Interval After Shock	Average Survival After Shock	Presence of Fibrinoid: Kidney	Spleen	Heart	Liver	Lung	Duration of Perfusion	Perfused Kidney	Non-perfused Kidney
4	6 hr.	20 hr.	3	3	2	2	3	3½ hr.	+	—
2	1 hr.	11 hr.	2	2	2	0	2	5 hr.	+	—
2	15 min.	1 hr.	0	2	1	0	2	1½ hr.	+	—
2	10 min.	1 hr.	0	0	0	2	1	1½ hr.	+	—
2	1 hr.	2 hr.	1	2	0	1	2	1 hr.	+	—
5	Control animals*		0	0	0	0	0	1 hr.	5—	5—

* All control animals and all recipients received 5 mg. of heparin prior to perfusion.

were noted to have cardiac fibrinoid and 50% revealed fibrinoid within the small arteries of the lung.

Control groups consisting of unprepared recipient animals transfused by unprepared donors or donors receiving a single intravenous injection of toxin exhibited no renal, splenic, hepatic, or pulmonary fibrinoid. A majority of these animals, like those in the other groups, were observed to have nonspecific myocardial hemorrhage and necrosis (Fig. 4). Of unexpected and unusual interest was the presence of fibrinoid within the substance of the cardiac valves (Fig. 5) in 33% of these animals. This material was morphologically and tinctorially indistinguishable from that seen in the valves of animals receiving two intravenous injections of toxin or toxin and sodium polyanethol sulfonate.

Isolated Renal Perfusion.—The results of these experiments are summarized in Tables 3 and 4.

Experiments were first conducted with the use of kidneys of recipient animals previously prepared by the intravenous injection of 2 cc. of 1:80 toxin 18 hours prior to perfusion. These kidneys were perfused with the blood of donor animals prepared and shocked with an equal amount and dilution of toxin. Both recipient and donor animals were given 5 mg. of heparin immediately prior to perfusion to prevent clotting within the cannulated vessels and polyethylene tubing. As is seen in Table 3, microscopic examination failed to reveal fibrinoid within the glomerular capillaries of any of these perfused kidneys, neither was its presence detected in the opposite nonperfused kidney.

Heparin is known to inhibit the deposition of glomerular fibrinoid.‡ To eliminate the possibility that this material was preventing the development of the renal lesions, unprepared recipient kidneys were perfused with the blood of donor animals prepared by simultaneous intravenous injections of 2 cc. of 1:1000 toxin and 10 mg. of sodium polyanethol sulfonate. Because of the active anticoagulant properties of sodium polyanethol

‡ References 5 and 6.

sulfonate, only the recipient animals were given 5 mg. of heparin prior to perfusion. In addition, the pump was utilized to effect more rapid and complete perfusion.

Reference to Table 4 shows that all of the perfused kidneys in this series were found to have fibrinoid within the glomerular capillaries. The appearance of this material was identical with that found in the glomerular capillaries of the early renal lesions following the administration of toxin or toxin and sodium polyanethol sulfonate. Typically the material appeared as a dense homogeneous, eosinophilic substance layered above the capillary endothelium, or completely occluding the capillary lumina, of the majority of glomeruli (Figs. 6, 7, and 8).

Because it was felt that the churning hemolytic action of the pump might contribute to the production of the renal lesions, a control group of unprepared recipient kidneys was perfused with the blood of unprepared donor animals. The negative results are summarized in Table 4.

COMMENT

Lesions identical with those occurring in animals given intravenous injections of meningococcal toxin or toxin and sodium polyanethol sulfonate can be produced by transfusion of recipient animals with appropriately treated donors. With the exception of less frequent renal involvement, the incidence of these lesions in prepared recipient animals is also comparable. The decreased incidence of renal lesions suggests the possibility that these changes might have occurred solely as a result of the preparatory injection of toxin. However, identical lesions were produced in unprepared transfused recipients.

Of considerable interest is the complete absence of renal lesions in transfused unprepared recipients. A number of factors may be responsible for this occurrence. During these experiments it was often noted on killing of the recipient animals, even as long as 24 hours after transfusion, that the kidneys were much smaller than normal and appeared pale and ischemic. It appears that reflex stimulation from procedures preceding and during transfusion results in marked constriction of the renal vascular bed for periods sufficiently long to prevent access of fibrinoid to the kidney and its deposition in the glomerular capillaries. Thomas has also observed a markedly lessened incidence of contralateral renal lesions in animals given two successive intravenous injections of meningococcal toxin after ligation of one renal artery.§

One would expect a similar circumstance to prevail in donor animals subjected to the same manipulation before and during transfusion. However, the donor animals were given one or more injections of toxin from one to four hours prior to transfusion. In these animals the endotoxin may have resulted in vasodilatation of the renal vessels, as observed by Smith,[9] counteracting the tendency to vasoconstriction during manipulation and transfusion. It was also necessary to observe directly the kidneys of a considerable number of donor animals prior to transfusion to insure the use of positive donors. As a result, in many instances only those donor animals in which a renal lesion was already present were used for transfusion purposes. It is also possible that the vascular damage observed to occur after a single injection of toxin is partly necessary for the deposition of fibrinoid within the glomerular capillaries. Although the 5 mg. of heparin given to each animal prior to transfusion is small in comparison with the amount found by Good and Thomas [8] to be necessary to prevent the deposition or fibrinoid in the glomeruli, its effect may have been an additional factor in the prevention of the renal lesions.

The presence of fibrinoid following transfusion in unprepared recipient animals offers considerable evidence for its transfer by way of the blood stream. The possibility cannot be ignored, however, that sufficient quantities of freely circulating toxin may be present in the blood of prepared donor animals two to four hours after its injection, so that transfer

§ Thomas, L.: Personal communication to the authors.

of this toxin may act in a manner similar to a single intravenous injection of toxin. The results of experiments in which unprepared recipient animals were injected with 2 cc. of donor blood containing an estimated dilution of 1:80 toxin per cubic centimeter are evidence that this does not occur. These results show that the lesions occurring in the prepared recipients in this procedure were the result of previous preparation alone and indicate that, even in such a small number of animals, the lower incidence of fibrinoid following a single injection of toxin can be observed. It is interesting that a prepared animal is able to detoxify such an abnormally large amount of toxin in two hours' time—an observation that speaks against the postulate that the first, or preparatory, injection of endotoxin may "block" the cells of the reticuloendothelial system so that the second injection may exert its effect(s) unimpeded.[10]

The presence of fibrinoid within the cardiac valves of unprepared recipients transfused with the blood of unprepared donor animals is of considerable interest. The possibility that this material is not identical with that found in the valves of animals given two intravenous injections of toxin is unlikely, when one considers its characteristic position and morphologic and tinctorial similarity to fibrinoid. There is, of course, the possibility that the material found in the valves of animals given endotoxin is not the same substance as that found in other sites, even though it is indistinguishable morphologically from this material. Previous experiments strongly suggest that this material is the same, however. Bilateral nephrectomy prior to two intravenous injections of toxin, or splenectomy prior to a single intravenous injection, greatly increases the incidence of fibrinoid within the heart valves and beneath the endothelium of the coronary arteries.‖

A possible explanation for the presence of fibrinoid within the valves of transfused normal animals may be found in the recent work of Thomas, Smith, and Von Korff.[11] These investigators have demonstrated the presence of a "heparin-precipitable protein" in the plasma of animals given a previous intravenous injection of endotoxin. The plasma of 20% of normal animals was also noted to contain a small amount of this material, which appears to be related to, or closely associated with, fibrinogen. They suggest that the heparin-precipitable protein may be a precursor for "fibrinoid-like material." It is possible that the valvular fibrinoid in the control animals represents the deposition of this material beneath the endothelium and within the substance of valves damaged during transfusion. That considerable hemodynamic alterations and damage to the fragile heart valves occur is shown by the frequent observation of intravalvular hemorrhages and extensive myocardial alterations in both experimental and control animals subjected to carotid-jugular cross transfusion.

Renal lesions identical with those occurring in animals given intravenous meningococcal toxin or toxin and sodium polyanethol sulfonate were produced only in the group of animals in which unprepared recipient kidneys were perfused with the blood of donors given simultaneous intravenous injections of toxin and sodium polyanethol sulfonate. The absence of renal lesions in the group in which donor animals were given two injections of toxin appears to be due to several factors. The fibrinoid deposits observed after the administration of toxin and sodium polyanethol sulfonate are much greater in amount than those observed after the administration of toxin alone.[8] This suggests that the amount of circulating fibrinoid is quantitatively much less after the latter procedure, and that during the relatively short time over which perfusion was carried out the small amount of heparin administered to the donor animals was sufficient to prevent the deposition of fibrinoid in the glomerular capillaries.

SUMMARY

By carotid-jugular cross transfusion, with the use of donor animals given meningococcal endotoxin or endotoxin and sodium poly-

‖ Brunson, J. G., and Gamble, C. N.: Unpublished observations.

anethol sulfonate, fibrinoid lesions of the kidney, spleen, liver, lungs, and heart were observed in the recipient animals. The incidence of the renal lesions, however, was much less than that observed in animals given intravenous meningococcal endotoxin or endotoxin and sodium polyanethol sulfonate. Control donor and recipient animals given no endotoxin or sodium polyanethol sulfonate developed valvular fibrinoid lesions in a high percentage of cases, but no other lesions were demonstrated. By isolated renal perfusion with the use of donor animals prepared with meningococcal endotoxin and sodium polyanethol sulfonate, renal lesions identical with those occurring in endotoxin- or endotoxin-and-sodium-polyanethol-sulfonate-treated animals were produced in all recipient kidneys.

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Systemic Fibrinoid Diseases

Similarity to Experimental Lesions in Rabbits

JOEL G. BRUNSON, M.D.
and
RICHARD L. DAVIS, B.A., Minneapolis

INTRODUCTION

In recent years renewed interest has been shown in certain diseases which, although varying in their clinical manifestations, have been grouped together as "connective tissue diseases." As proposed by Klinge in 1933,[1] the group included rheumatic fever, rheumatoid arthritis, periarteritis nodosa, and dermatomyositis. In 1938 Masugi and Yä-Shu [2] added scleroderma, and in 1942 Klemperer and co-workers [3] placed lupus erythematosus in this category. The latter authors also introduced the term "diffuse collagen disease" to emphasize the "systemic involvement of the entire connective tissue of the human body" in each of these diseases. Each of the above writers also stressed the presence of "fibrinoid degeneration" in these diseases and considered this material to be the most prominent alteration in the connective tissue. Klemperer [3] described "fibrinoid degeneration" as a "descriptive morphologic term indicating certain well-defined optical and tinctorial alterations in the collagenous fibers and ground substance," and he considered "fibrinoid degeneration and collagen sclerosis" as the "morphologic expression of different phases of a disturbed colloidal collagen system."

The concept of these diseases [4] is therefore based largely on the occurrence of fibrinoid in lesions of the cardiovascular system and connective tissue, and it is generally agreed that any of these diseases may be associated with the development of widespread lesions affecting many organs and tissues. For reasons to be discussed later, the disease known as thrombotic thrombocytopenic purpura should also be included in this group.

Altshuler and Angevine [5] performed histochemical studies on the fibrinoid material occurring in these diseases and found it to have similar tinctorial properties in each type. They suggested that it represented an alteration in the "ground substance." The similarity of its morphologic and tinctorial properties suggests, in addition, that its chemical composition in each case may be similar or identical.

Clinically, these diseases can usually be separated, but the wide variation in symptomatology occasionally creates difficulty in recognition of any one specific entity. It is perhaps a less well-known fact that these diseases also overlap pathologically and, indeed, occasionally give rise to alterations which suggest an entirely different diagnosis.

As indicated in previous papers,* the generalized Shwartzman phenomenon is characterized pathologically by the occurrence of widespread fibrinoid lesions affecting the cardiovascular system, and this phenomenon provides a useful model for studying the origin of this material and the development of lesions associated with its presence. The purpose of this paper is therefore twofold: first, to point out the basic

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From the Department of Pathology, University of Minnesota Medical School.

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* References 6, 7, and 8.

morphologic similarities between the "collagen diseases" and, second, to illustrate the similarity between the lesions in these diseases and those occurring in the generalized Shwartzman phenomenon.

MATERIAL

The material included in this report consists of six recent cases of fibrinoid disease studied in the Department of Pathology of the University of Minnesota and of material obtained from experimental procedures carried out in rabbits. The latter consists of selected slides from animals given meningococcal endotoxin, alone or in conjunction with certain acidic polymers such as sodium polyanethol sulfonate (Liquoid-Roche), the sodium salt of the ester of polyvinyl alcohol and polysulfonic acid (PVAS-Roche), or dextran sulfate. The methods of preparation and administration of these materials and a detailed account of the morphologic changes which occur after their administration have been described in previous papers.†

PRESENTATION OF MATERIAL

CASE 1.—A 3-year-old white boy was admitted to University Hospitals April 17, 1954, with a history of fever, anorexia, malaise, and skin rash of three weeks' duration and a heart murmur of one day's duration. His illness began one month prior to admission with an upper respiratory infection associated with enlarged cervical lymph nodes. This improved in three or four days but was followed one week later by a skin rash, fever, and abdominal and left leg pain. Three days later the left knee became swollen, and physical examination by his local physician showed the following: nonpruritic urticarial-like eruption on the abdomen, lower back, and thighs; slight injection of the throat; enlarged cervical nodes, and slight left limp. No cardiac murmurs were heard at this time. His hemoglobin was 11 gm. per 100 cc., and the white blood cell count, 13,700 with 60% neutrophiles and 5% eosinophiles. One day prior to admission a loud systolic murmur was heard.

On admission he appeared acutely ill, pale, and apprehensive, with blood pressure 110/55, pulse rate 120, respiration 32, and temperature 102.6 F. There was a nonpruritic urticarial eruption over the abdomen, back, chest, arms, and upper thighs. The tonsils were enlarged and injected, and the throat appeared inflamed. Bilateral anterior cervical and submaxillary nodes were felt. The lungs were clear. The heart was enlarged to the left, and there was an apical systolic thrill. Coarse, Grade 3 systolic and Grade 1 diastolic murmurs were heard at the apex, and both were transmitted throughout the chest. The liver was palpable three finger-breadths below the right costal margin, and it was soft and nonpulsating. The left wrist was painful on motion but showed no swelling.

Laboratory studies showed the hemoglobin to be 10.6 gm. per 100 cc., and a white cell count of 11,650 with 80% neutrophiles. The sedimentation rate was 101 mm. per hour and the antistreptolysin O titer 625 U/cc. Urinalysis showed 1+ albumin and 1+ sugar. An electrocardiogram showed sinus tachycardia and prolongation of the P-R interval. Chest x-rays showed generalized cardiac enlargement. Blood cultures were sterile, and nose and throat cultures grew no pathogens. Corticotropin (75 mg. every 12 hours), digitoxin, penicillin, aminophylline, and ascorbic acid (500 mg. daily) were administered, but the patient's condition deteriorated and he died in pulmonary edema two days after admission.

Autopsy.—The lungs weighed 250 and 220 gm. and were grossly edematous. The heart weighed 140 gm., was dilated and flabby, and showed areas of fibrinous pericarditis anteriorly. The atrial surfaces of the tricuspid and mitral valves showed numerous firm, opaque, grayish-white nodules approximately 1 mm. in diameter, and the valve leaflets appeared slightly thickened. No gross defects were noted in the myocardium. The liver weighed 520 gm. and appeared congested. The remaining organs appeared grossly normal. Postmortem blood culture was sterile.

The significant microscopic findings were as follows: a severe pneumonitis characterized by edema, polymorphonuclear cellular infiltration, and alveolar hyaline membrane formation. Occasional pulmonary arteries showed endothelial proliferation. The heart showed acute diffuse rheumatic changes with numerous Aschoff bodies in the myocardium and acute mitral and tricuspid valvulitis with fibrinoid deposition (Figs. 1 and 5). The liver and spleen showed passive congestion.

CASE 2.—A 40-year-old white woman was first admitted to University Hospitals in February, 1953, with a three-year history of lupus erythematosus. The onset of the disease had been in late 1950, with a butterfly rash over the bridge of the nose. She was treated with intramuscular injections of bismuth with some improvement. In the fall of 1951 the rash reappeared and she felt "run down." During the summer of 1952 she developed swelling and aching of the hands and a mild migratory arthralgia. She was seen at the Mayo Clinic in November, 1952, and was placed on cortisone therapy (25 mg. daily).

† References 6, 7, and 8.

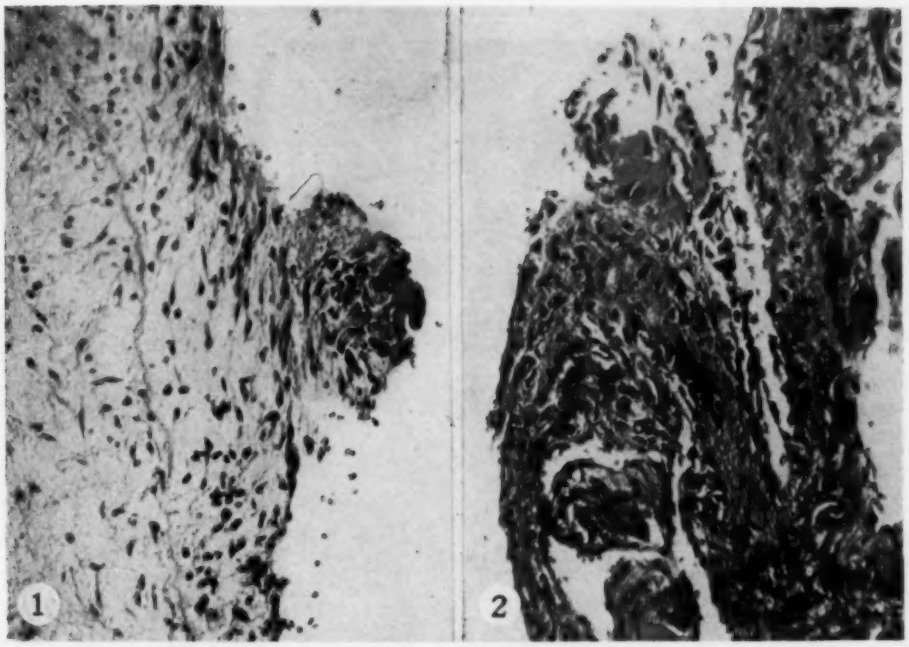
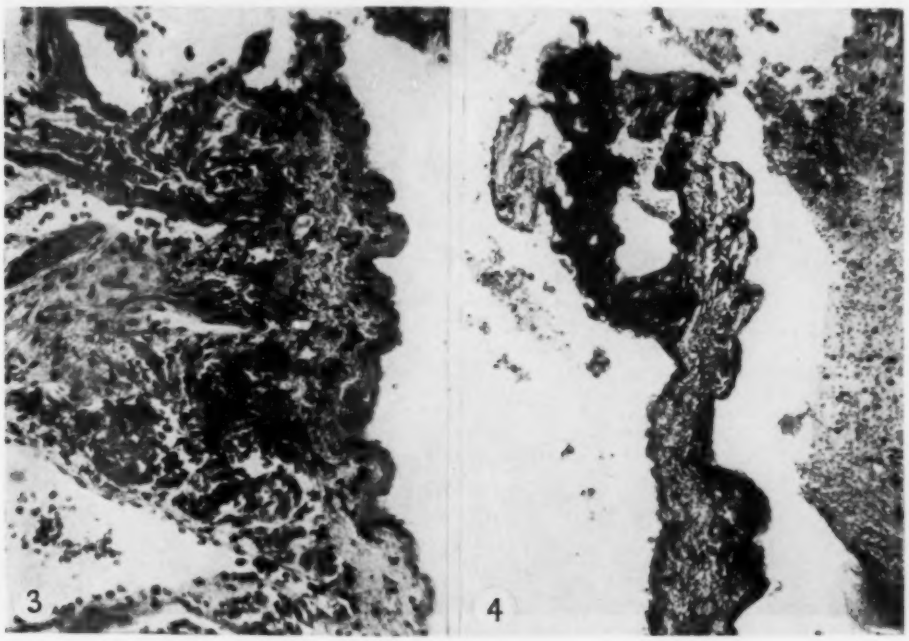


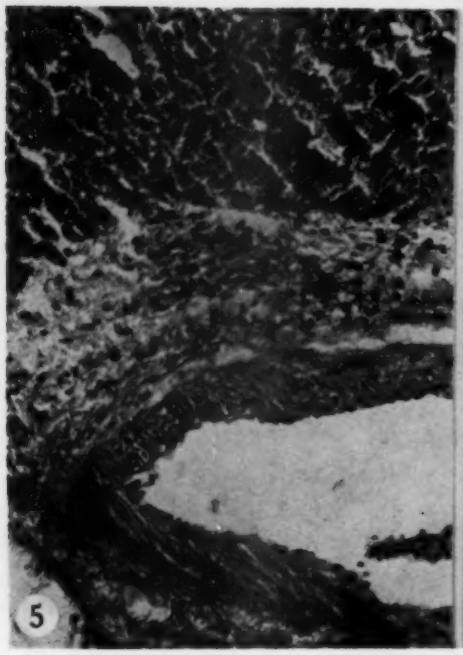
Fig. 1 (Case 1).—Section through a nodule on the mitral valve showing fibrinoid on the free margin with an accompanying cellular reaction in the substance of the valve. Hematoxylin and eosin; × 175.

Fig. 2.—Section from the mitral valve of a rabbit given intravenous meningococcal endotoxin and sodium polyanethol sulfonate. The free margin shows an accumulation of fibrinoid and there is an underlying cellular reaction. Hematoxylin and eosin; × 125.

Fig. 3.—Section from the heart of a rabbit given two intravenous injections of meningococcal endotoxin. A dense accumulation of fibrinoid is present along the pericardial surface, and there is marked hemorrhage and inflammatory reaction in the underlying muscle. Hematoxylin and eosin; × 175.

Fig. 4.—Section from the mitral valve of a rabbit given two intravenous injections of meningococcal endotoxin. Stained by the periodic acid-Schiff method. Note the intensely Schiff-positive material beneath the endothelium of the valve and also projecting from its free margin; × 125.





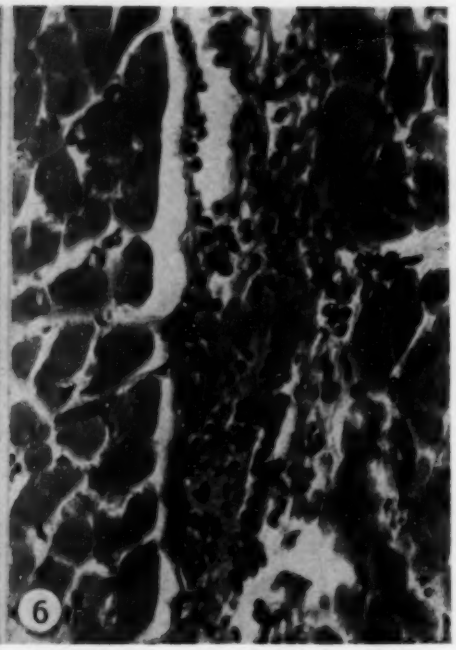
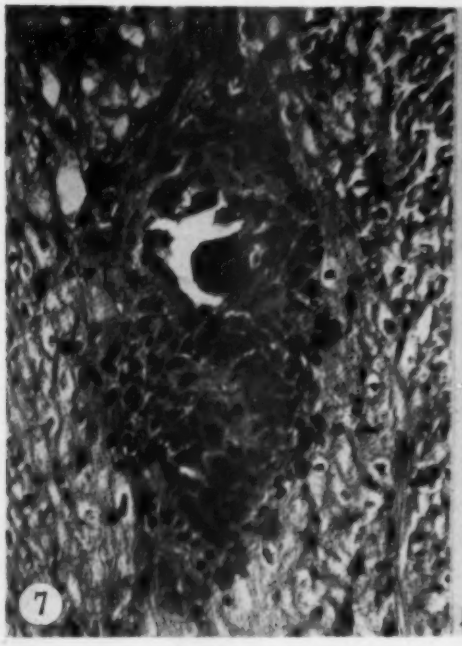


Fig. 5 (Case 1).—Section from the myocardium showing a perivascular Aschoff body. Hematoxylin and eosin; × 175.

Fig. 6 (Case 4).—Section from the myocardium showing interstitial accumulation of mononuclear cells and fibrinoid about a small coronary vessel. Hematoxylin and eosin; × 350.

Fig. 7.—Section from the myocardium of a rabbit given two intravenous injections of meningococcal endotoxin showing a fusiform collection of mononuclear cells about an intramural coronary artery. Hematoxylin and eosin; × 400.

Fig. 8.—Intramural coronary artery with a focal subendothelial accumulation of fibrinoid which projects into the lumen of the vessel. From a rabbit given an intravenous injection of meningococcal endotoxin and sodium polyanethol sulfonate. Hematoxylin and eosin; × 400.



After her discharge from the Clinic she remained in bed and became progressively weaker. Her local physician gradually increased the dosage of cortisone to 200 mg. daily.

On admission physical examination showed a red-brown, scaling, crusty dermatitis over the face, ears, neck, palms, and upper chest, but no other significant abnormalities. Laboratory studies showed a constant albuminuria (1 to 2+), leucopenia (850 to 8200), anemia (hemoglobin 3.9 to 11.9 gm. per 100 cc.), and an elevated sedimentation rate (52 to 162 mm. per hour). Three tests for L. E. cells were positive.

While the patient was on cortisone therapy, she developed a psychotic reaction which improved with gradual reduction in dosage of the drug, and she was discharged in October, 1953, after a period of approximately eight months.

Her second and final admission occurred Dec. 30, 1953, with complaints of a productive cough, general malaise, weakness, and weight loss, of approximately four weeks' duration. She had been previ-

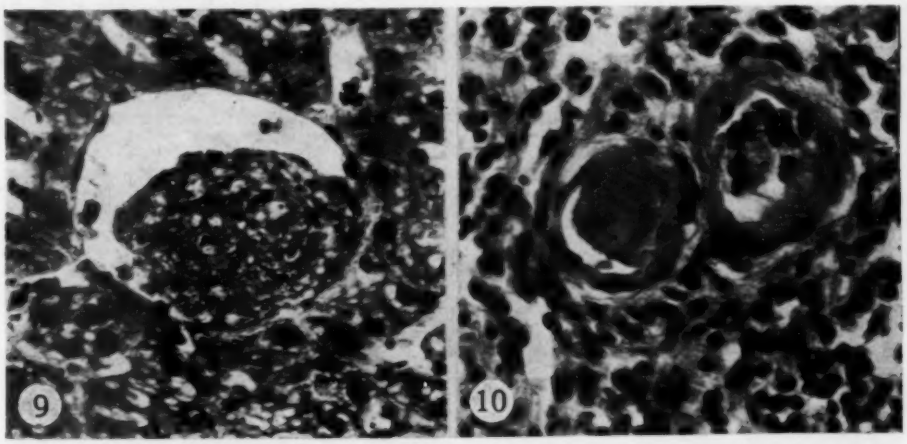
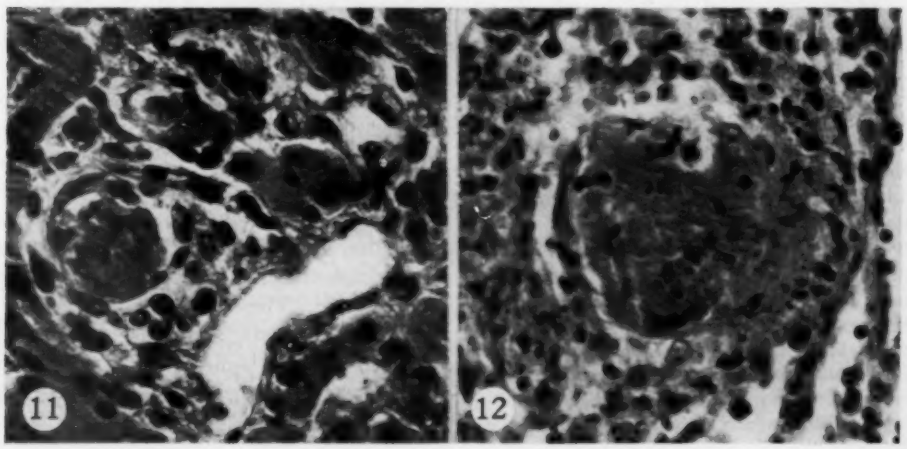


Fig. 9.—Section from the liver of a rabbit given two intravenous injections of meningococcal endotoxin. Note fibrinoid thrombus within the lumen of an efferent vein. Hematoxylin and eosin; × 400.

Fig. 10 (Case 2).—Section from a lymph node showing fibrinoid deposition in two arterioles. In one the material involves almost the entire media of the vessel, while in the other it is partially covered by endothelium and projects into the lumen of the vessel, simulating the appearance of vessels in thrombotic thrombocytopenic purpura. Hematoxylin and eosin; × 350.

Fig. 11 (Case 6).—Thrombotic thrombocytopenic purpura. Section from the liver showing the accumulation of fibrinoid in two arterioles. Hematoxylin and eosin; × 350.

Fig. 12 (Case 5).—Section from a lymph node showing an arteriole with fibrinoid deposited in its wall and within its lumen. Hematoxylin and eosin; × 450.



ously hospitalized in her home community for a period of four days, with a temperature of 104 F.

Admission examination showed: pulse rate 132 and irregular; temperature 102.6 F; respiration 24, and blood pressure 130/100. There was left-sided cardiac enlargement, irregular rhythm, and a Grade 2 apical systolic murmur transmitted over the entire precordium. Inspiratory wheezes were heard in the right upper chest. The liver was palpable 3 to 4 cm. below the right costal margin, and the spleen was questionably palpable. Bilateral upper quadrant abdominal tenderness was present on palpation.

The hemoglobin was 8.0 gm. per 100 cc., and the white blood cell count, 4950 with 88% neutrophiles. Urinalysis showed 1+ albumin. Total serum proteins were 5.5 gm. per 100 cc., with globulin 4.0 per 100 cc. Venous pressure was 8.5 cm. citrate, and circulation time arm-to-tongue was 30 seconds. Chest x-rays showed right upper lobe atelectasis probably due to an inflammatory process, but no fluid, and electrocardiogram showed premature ventricular contractions. During the next two days the patient became progressively weaker and died Jan. 2, 1954.

Autopsy.—Bilateral pleural effusion (550 cc.), pericardial effusion (450 cc.), and ascites (150 cc.) were present. The heart weighed 350 gm., and there was slight thickening of the mitral valve but no other gross changes. The lungs showed areas of bronchopneumonia and atelectasis. The liver weighed 1550 gm. and showed mild fatty changes. The spleen weighed 190 gm. and appeared fibrous on cut section. The kidneys weighed 150 gm. each and showed numerous subcapsular petechial hemorrhages. Postmortem blood culture was sterile.

Significant microscopic findings: Many of the renal glomerular capillaries were occluded by dense, hyaline, refractile material, which stained intensely red with the periodic acid-Schiff stain, homogeneous purple to orange-purple with phosphotungstic acid hematoxylin, and blue with toluidine blue (Fig. 23). In other areas this fibrinoid material was closely applied to the capillary basement membranes producing the typical "wire loops" of lupus erythematosus. Similar material was observed in the arterioles of the spleen and the lymph nodes (Fig. 10) and was associated with areas of hemorrhage and necrosis. The myocardium showed areas of fibrosis and the mitral valve was thickened and hyalinized, but no acute cardiac changes were noted. The lungs showed areas of fibrosis, atelectasis, and bronchopneumonia. No hematoxylin bodies were observed, and no periarterial fibrosis of the splenic arteries was noted.

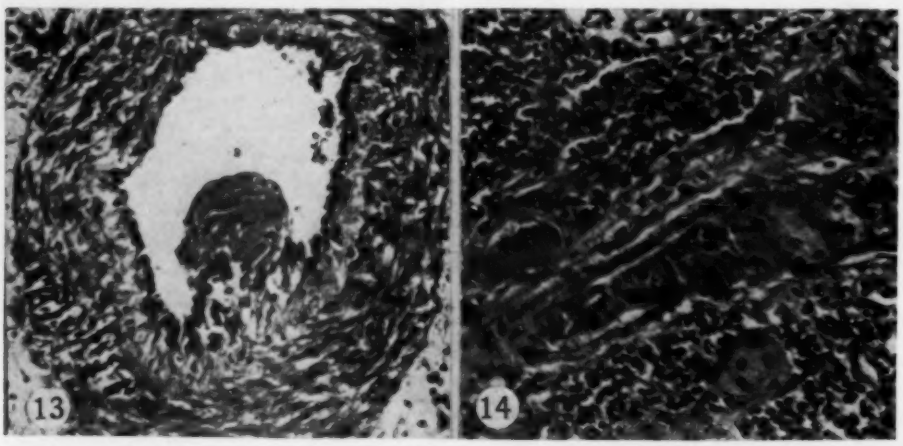


Fig. 13.—Pulmonary artery from a rabbit given two intravenous injections of meningococcal endotoxin with fibrinoid projecting from the wall of the vessel. The luminal surface of the fibrinoid is covered by endothelium, and there is endothelial proliferation around the site of attachment. Hematoxylin and eosin; × 200.

Fig. 14 (Case 6).—Section from the thymus showing two arterioles with fibrinoid deposition. In the upper vessel, cut transversely, the material projects from the wall of the arteriole and is covered by endothelium. In the lower vessel, cut longitudinally, the fibrinoid is intraluminal and contains considerable nuclear debris. Hematoxylin and eosin; × 200.

CASE 3.—A 76-year-old white woman was admitted to University Hospitals for the sixth time Jan. 25, 1954, with complaints of dysphagia, sensation of choking, poor vision, exertional dyspnea, dry cough, weakness, fatigue, anorexia, constipation, occasional stiffness and tenderness of the fingers, and a "tight feeling" in her face. Her first, third, and fourth admissions (1947, 1950, and 1952) had been for a bleeding duodenal ulcer which was treated medically. The second and fifth admissions (1949 and 1953) were for hypertensive cardiovascular disease. During the latter two admissions the highest recorded blood pressures were 180/80 and 198/84, respectively. At the time of her admission in November, 1953, she had been in congestive failure and was digitalized, but her condition improved very little.

On admission physical examination showed: blood pressure 170/95; temperature 99 F; pulse rate 88, and respiration rate 32. Scattered rales were heard in both lung bases. The heart was enlarged to the left and had a normal sinus rhythm with occasional premature ventricular contractions. A thickening of the skin and subcutaneous tissues of the fingers and toes was noted. The hemoglobin was 11.7 gm. per 100 cc., white blood cell count was 13,250 to 23,000 with 78% to 89% neutrophiles, and urinalysis showed 2+ albumin. A blood culture was sterile. The prothrombin time was 14.7 seconds, with a control of 12.5 seconds. Stool benzidine and guaiac reactions were 4+. X-Ray examinations showed a lack of esophageal tone with narrowing of the distal end, a deformed duodenal bulb, and diffuse fibrosis of both lungs. Admission electrocardiogram showed no abnormalities, but later showed a wandering pacemaker. Approximately 10 days after admission the patient developed bronchopneumonia, and 5 days later pulmonary edema developed and she died.

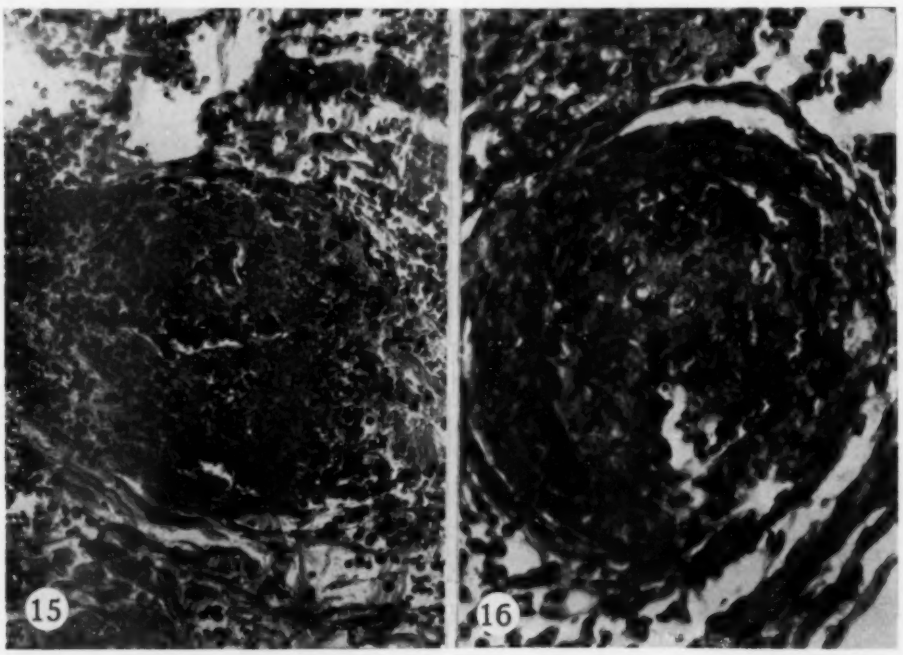


Fig. 15 (Case 5).—Vessel from the thyroid gland showing complete replacement of its wall by fibrinoid, with a surrounding acute inflammatory reaction. Hematoxylin and eosin; × 200.

Fig. 16.—Intestinal artery from a rabbit given meningococcal endotoxin and sodium polyanethol sulfonate. The media of the vessel is largely replaced by fibrinoid, which is associated with an intraluminal thrombus and an acute inflammatory reaction. Hematoxylin and eosin; × 350.

Autopsy.—Diffuse pulmonary fibrosis and bronchopneumonia. Pericardial effusion (150 cc.). The heart was of normal size but showed severe coronary atherosclerosis and myocardial fibrosis. The esophagus showed a postmortem rupture with liberation of gastric contents into the left pleural cavity. The first portion of the duodenum showed numerous small ulcerations. The liver and spleen appeared normal. The kidneys were small (90 gm.)

and markedly pitted. The skin was deeply pigmented and thickened over the abdomen, anterior chest, and extremities.

Microscopic findings included the following: The renal arteries and arterioles showed severe hyaline sclerosis with occasional subendothelial fibrinoid deposition. Similar material was also present in the lumen of afferent arterioles (Fig. 28) and in many of the glomerular capillaries. The latter lesions were identical with the "wire loops" of lupus erythematosus (Fig. 24). Extensive pulmonary fibrosis and bronchopneumonia were present, and occasional pulmonary arterioles were occluded by fibrinoid material similar to that observed in the kidneys (Fig. 19). There was a diffuse myocardial fibrosis with occasional areas of perivascular mononuclear cellular reaction, and severe coronary atherosclerosis. The mitral valve was thickened and hyalinized but showed no acute changes. The liver showed passive congestion and areas of acute focal necrosis with neutrophilic reaction. The duodenum showed multiple healed ulcerations; the esophagus, a postmortem rupture. The skin showed changes typical of scleroderma.

Case 4.—An 11-year-old white girl was admitted to University Hospitals Feb. 5, 1954, with a diagnosis of disseminated lupus erythematosus. History dated the onset of her illness to October, 1953, at which time a rash appeared on the bridge of the nose. This failed to respond to local therapy and two weeks later, when she became lethargic, she was hospitalized elsewhere. A diagnosis of acute disseminated lupus erythematosus was made from bone marrow preparations, and she was placed on cortisone therapy. After her discharge occasional febrile episodes occurred and she developed peripheral edema and ascites. Hypertension (to 190 systolic) and headaches later developed, and she was placed on a low-sodium diet by her local physician. Four days prior to admission she developed a firm puffiness of the face and neck, became listless, and began vomiting.

On admission the patient appeared acutely ill, and there was a generalized edema, more pronounced around the neck. Blood pressure was 156/88; pulse rate, 80; temperature, 99.2 F, and respiration rate, 32. A maculopapular rash was present over the cheeks and upper eyelids. The lungs were clear. The heart was enlarged to the left, and there was a rough, Grade 3 systolic murmur, best heard at the apex. The abdomen showed signs of free fluid, and there was generalized and rebound abdominal tenderness. She also appeared somewhat disoriented.

Hemoglobin on admission was 8.9 gm. per 100 cc.; white blood cell count, 11,450 with 87% neutrophiles. The sedimentation rate was 131 mm. per hour, bleeding time 3 minutes 30 seconds, and clotting time 4 minutes 30 seconds. Urinalysis showed 2+ albumin and 2+ red blood cells in the sediment.

During the night following admission the patient became unconscious and cyanotic, but she responded to stimulants and was placed in oxygen. Two hours later, however, her breathing became labored and she died in acute pulmonary edema.

Autopsy.—Ascites (100 cc.), bilateral pleural effusion (75 cc.), and pericardial effusion (50 cc.) were present. The heart appeared enlarged and weighed 340 gm. The mitral valve appeared thickened. The lungs weighed 250 and 220 gm. and showed acute edema. The liver weighed 1190 gm. and the spleen 150 gm., but neither showed any gross changes. The kidneys weighed 200 gm. each and showed numerous petechial hemorrhages. Postmortem blood culture was sterile.

Microscopically, a variety of changes was observed. The renal arterioles showed changes typical of periarteritis nodosa (Fig. 17), and the glomerular capillaries showed the presence of fibrinoid, which in some areas produced a "wire loop" lesion and in others appeared to lie free in the lumen (Fig. 25). Other glomeruli appeared identical with the subacute phase of glomerulonephritis. Lesions of periarteritis nodosa were observed in other organs, particularly the heart and liver (Fig. 18), and this seemed to be the predominant lesion. However, the mitral valve showed an acute cellular reaction without fibrinoid deposition, and the myocardium showed numerous areas of focal necrosis and cellularity that resembled Aschoff bodies (Fig. 6). Sections from the brain showed nonspecific degenerative changes. No hematoxylin bodies were noted, and the splenic arteries appeared normal.

Case 5.—A 32-year-old white woman was admitted for the first time to University Hospitals April 22, 1950, for study of liver and kidney malfunction. She gave a history of easy bruising,

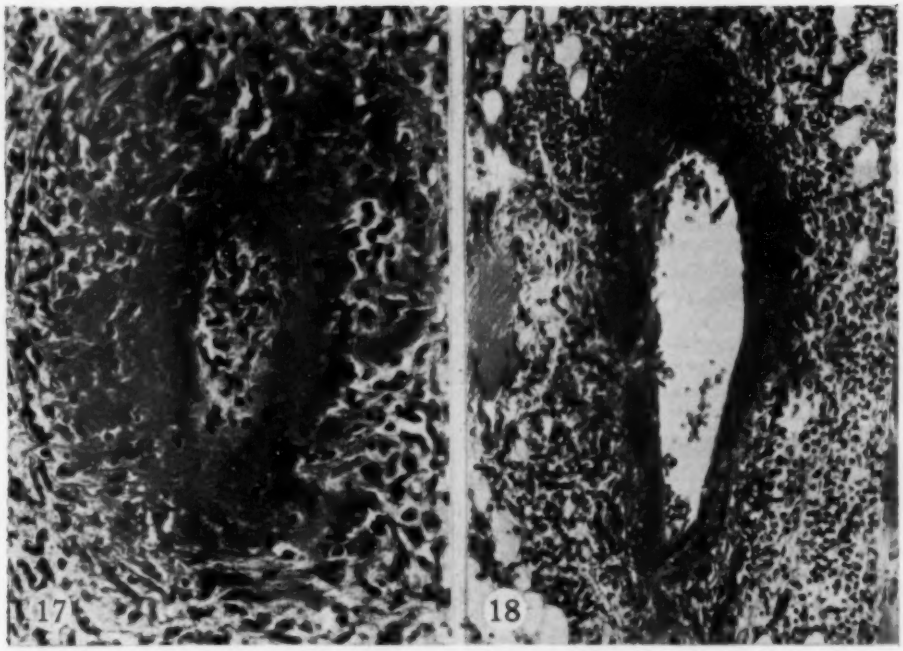
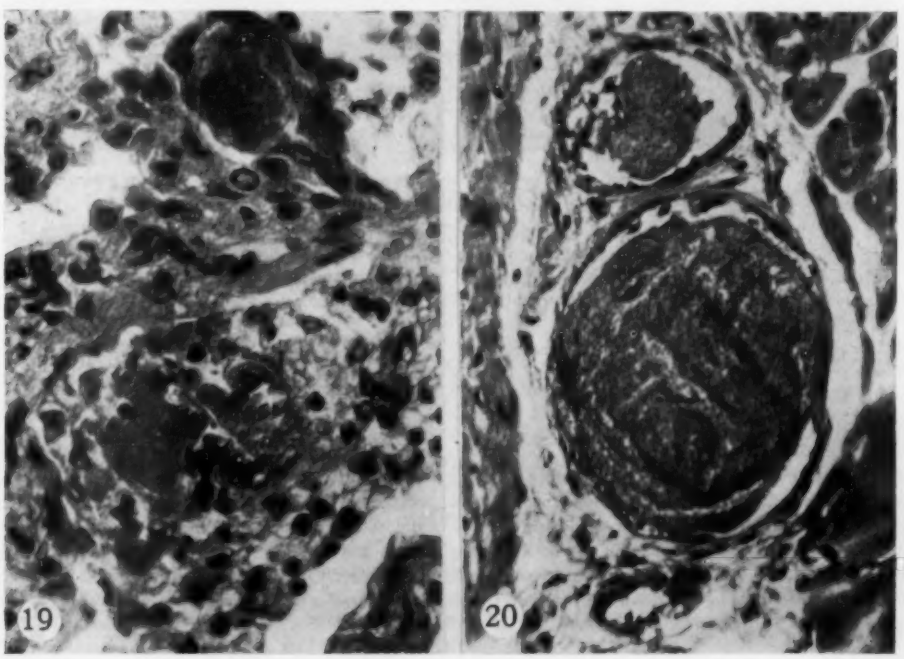


Fig. 17 (Case 4).—Renal arteriole showing complete replacement of its media by fibrinoid with periadventitial accumulation of this material, and a mononuclear cellular reaction. Hematoxylin and eosin; × 350.

Fig. 18 (Case 4).—Pericardial coronary artery with segmental fibrinoid deposition and surrounding acute inflammatory reaction. Hematoxylin and eosin; × 150.

Fig. 19 (Case 3).—Pulmonary arterioles showing the presence of fibrinoid within their lumina and in their walls. No inflammatory reaction is present. Hematoxylin and eosin; × 500.

Fig. 20.—Intramural coronary artery from a rabbit given two intravenous injections of meningococcal endotoxin. Note granular appearance of the thrombus, its continuity with the vessel wall, and the absence of inflammatory reaction. Hematoxylin and eosin; × 400.



multiple food allergies, intermittent eczema since birth, and epistaxis with menstruation. During her first pregnancy in 1946 a positive serology was noted; this had been negative six months previously. She also developed mild ankle edema during this pregnancy and, after delivery, complained of menorrhagia and easy fatigability. During the third trimester of pregnancy in 1948 albuminuria and peripheral edema developed, and the former persisted (4+) to the time of hospital admission. In October, 1949, a 4+ cephalin-cholesterol flocculation and an increased prothrombin time were noted.

Admission physical examination showed an eczematoid eruption on the cheeks, nose, and forehead, multiple bruises and ecchymotic areas especially marked on the lower extremities, and a soft apical systolic murmur.

Laboratory studies showed a 1 to 2+ albuminuria; hemoglobin of 13.8 gm. per 100 cc., white blood cell count of 6800 with 81% neutrophiles; platelet count of 168,000; bleeding time of 3 minutes, and capillary clotting time of 13 minutes. The sedimentation rate was 72 mm. per hour, stool benzidine reaction 4+, and stool guaiac reaction 1+. Lee-White clotting time was 101 minutes in the third tube, and the cuff test was strongly positive. The patient was discharged after a period of six days, to return later for steroid therapy, although no specific diagnosis had been established. Tests for L. E. cells had been negative.

The patient was next admitted May 18, 1950, for therapy. Physical examination on this admission was essentially the same as previously. Laboratory studies showed a persistent 3+ albuminuria; white blood cell count of 10,500 with 80% neutrophiles, and prothrombin time of 26.5 seconds, with a control of 12.5 seconds. Total serum proteins were 6.1 gm. per 100 cc. with 3.6 gm. per 100 cc. globulin. The Wassermann reaction was 4+, and the V. D. R. L., weakly positive. Tests for L. E. cells were negative. She was placed on corticotropin therapy but showed no improvement after a period of eight days and was discharged.

The third admission occurred July 9, 1951, when the patient was in the second trimester of her third pregnancy. At this time she gave a history of weight gain of 20 lb. (9.1 kg.) in association with some nausea and vomiting. Physical examination (except for enlarged uterus) was essentially the same as that on previous admission, and the blood pressure was

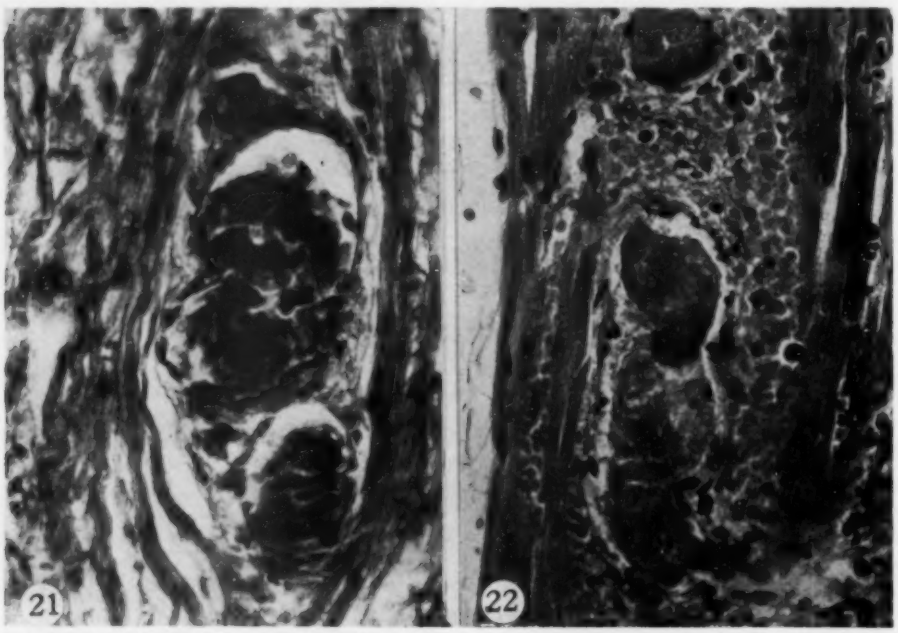


Fig. 21 (Case 6).—Section from the myocardium showing intraluminal and subendothelial fibrinoid in an intramural coronary artery. Periodic acid-Schiff stain; × 350.

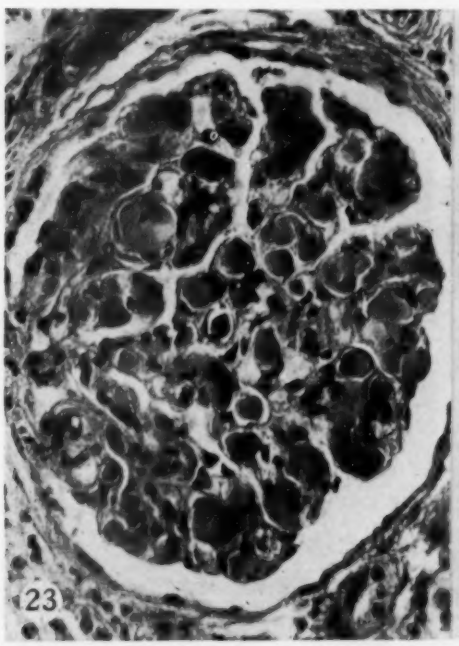
Fig. 22.—Section from the myocardium of a rabbit given four daily intravenous injections of meningococcal toxin and sodium polyanethol sulfonate. The animal died on the fifth day. Note homogeneity of the fibrinoid within the lumen of the coronary artery, the surrounding hemorrhage, and the absence of inflammatory reaction. Hematoxylin and eosin; × 350.

within normal limits. Moderate pitting edema of the legs was noted. Urinalysis showed 2+ albumin, and the hemoglobin was 10.6 gm. per 100 cc. The white blood cell count was 4350 with a normal differential. Platelets were 144,000, and sedimentation rate was 164 mm. per hour. Total serum proteins were 5.4 gm. per 100 cc., with globulin 3.3 gm. per 100 cc. She was discharged three days after admission, to be followed in the outpatient department.

The fourth and final admission occurred June 6, 1954, after the onset of acute abdominal pain with tenesmus and diarrhea six days previously. On examination the blood pressure was 130/80, temperature 99 F, and pulse rate 100. The lungs were clear, and cardiac examination showed a soft apical systolic murmur. Abdominal examination revealed diffuse and rebound tenderness, and pelvirectal examination showed a boggy resistance and slight tenderness in the cul-de-sac. The bowel sounds, normal on admission, became absent three hours later.

Admission laboratory studies showed a hemoglobin of 11.3 gm. per 100 cc., white blood cell count 7300 with 73% neutrophiles, and blood urea nitrogen of 33 mg. per 100 cc. Urinalysis showed 2+ albumin, and 3+ red blood cells in the sediment. Total serum proteins were 5.1 gm. per 100 cc. During the week following her admission she progressively deteriorated and died June 13, 1954.

Autopsy.—Numerous ecchymoses, 500 cc. of bloody fluid in the abdomen, and 800 cc. of dark bloody fluid in each pleural cavity were present. The heart was of normal size but showed areas of fibrosis approximately 0.5 cm. in diameter. The lungs showed atelectasis of both lower lobes. Multiple subserosal hemorrhages were noted throughout the intestinal tract, occasional hemorrhages extending through the wall of the bowel. Both adrenals were markedly enlarged and appeared almost entirely replaced by hemorrhage. The liver weighed 1790 gm. and showed large areas of hemorrhage and necrosis. The spleen weighed 340 gm. and was soft on cut section. Each kidney weighed 230 gm. and showed multiple subcapsular hemorrhages. The uterine cavity was filled with blood. Postmortem blood culture was sterile.



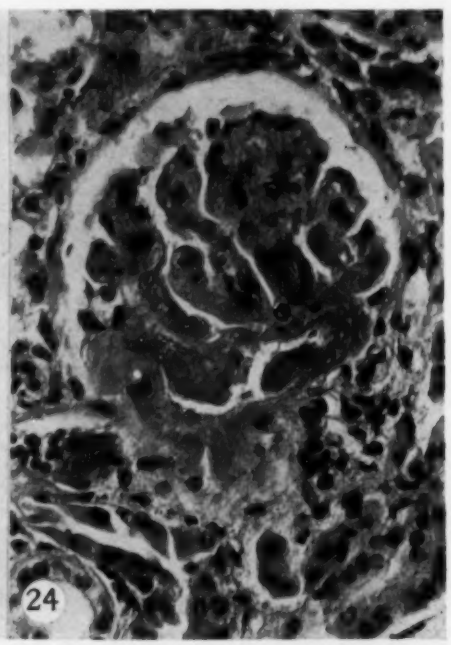


Fig. 23 (Case 2).—Glomerulus with accumulation of fibrinoid within the lumina of the capillaries. In a few vessels the material is applied closely to the basement membrane, but most of it appears to lie free in the lumen. Hematoxylin and eosin; × 350.

Fig. 24 (Case 3).—Glomerulus with accumulation of fibrinoid which lies within the lumina of some of the capillary loops and in others is applied to the basement membrane, producing a "wire loop" appearance. Hematoxylin and eosin; × 350.

Microscopically, many "wire loops" were noted in the renal glomerular capillaries (Fig. 29). The heart showed areas of scarring and areas of fresh necrosis and

neutrophilic cellular reaction. The mitral valve showed hyaline thickening but no acute changes. The liver showed diffuse hemorrhage and necrosis, and the vessels and sinusoids contained thrombotic material which was distinctly eosinophilic, homogeneous, and refractile. This material gave the usual staining reactions of fibrinoid and in some vessels was noted to lie beneath the endothelium. Focal areas of hemorrhage and necrosis were noted in the spleen and the lymph nodes, and the small arteries in close proximity to these areas were occluded by fibrinoid material (Fig. 12). The intestinal lesions consisted of diffuse hemorrhage through the muscular coat, and many of the arteries in the wall of the intestine were occluded by material similar to that described in the liver, spleen, and lymph nodes. In sections from other organs occasional arteries showed fibrinoid replacement of most of the vessel wall with surrounding acute cellular reaction, producing a picture identical with that of periarteritis nodosa (Fig. 15). Sections from both adrenals showed the parenchyma to be almost entirely replaced by hemorrhage. Sections from the brain showed no changes.

Case 6.—A 7-month-old white boy was first admitted to University Hospitals July 20, 1953, at the age of 3 months. At that time there was a history of intermittent bloody-mucous stools since birth. Physical examination showed a mucopurulent discharge from the eyes, a right cervical lymph node enlargement, normal heart and lungs, palpable spleen tip, seborrheic rash over the scalp, and a maculopapular rash on the neck and abdomen. During this admission laboratory studies showed a constant macrocytic hypochromic anemia (hemoglobin 6.2 to 10.3 gm. per 100 cc.), an intermittent eosinophilia (2% to 16%), and persistent neutrophilia. The sedimentation rate was elevated (111 to 128 mm. per hour), and there was a persistent

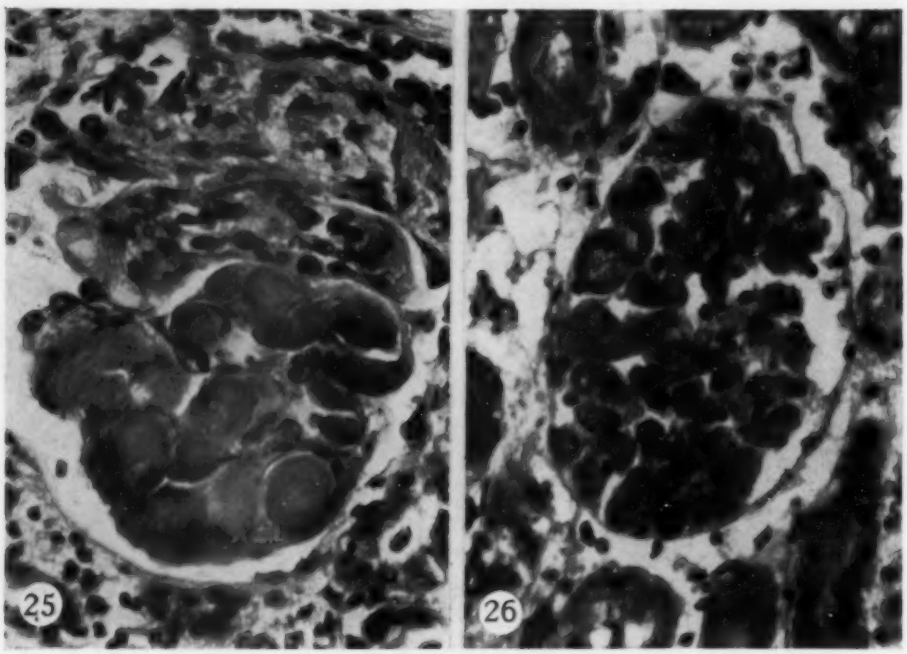


Fig. 25 (Case 4).—Glomerulus with deposits of fibrinoid within the lumen and adjacent to the basement membranes of the capillary loops. Hematoxylin and eosin; × 450.

Fig. 26.—Glomerulus from a rabbit given an intravenous injection of meningococcal toxin and sodium polyanethol sulfonate. The fibrinoid is closely applied to the capillary basement membranes, producing a lesion similar to the "wire loops" of disseminated lupus erythematosus. Hematoxylin and eosin; × 400.

thrombocytopenia (28,000 to 110,000). The cuff test was strongly positive, and the bleeding time was prolonged (10 minutes 30 seconds). The stools were negative for ova and parasites, and were negative to guaiac but were consistently positive to benzidine. Liver function studies were essentially normal. Extensive work-up including cultures of blood and bone marrow failed to reveal a definite diagnosis, and the patient was discharged Sept. 9, 1953.

The patient was next admitted Nov. 2, 1953, with a history of occasional bleeding episodes from the skin rash. Physical examination showed spread of the seborrhea to the face and neck and open areas of eczema on the extremities. A mild generalized enlargement of the lymph nodes was noted, and a short, Grade 1 systolic murmur was heard along the left sternal border. The liver edge was felt 4 cm. below the right costal margin, and the spleen tip was again felt. The abdomen and neck had an "old purpuric appearance."

Laboratory studies were essentially the same as on previous admission. A biopsy of the skin was nondiagnostic, and the lesions improved with dry therapy. He was discharged Nov. 28, 1953, to be followed in the outpatient department.

The third and final admission occurred Dec. 8, 1953, and was precipitated by a two-day episode of vomiting. The patient appeared acutely and chronically ill at this time, with a temperature of 102.6 F and respirations of 64 per minute. There were numerous fresh and old petechiae over the body. The fundi were normal, and there was moderate resistance to neck flexion without gross neck or back rigidity. The liver was felt 6 to 8 cm. below the costal margin, and the spleen was palpable. While a lumbar puncture was being done, he suddenly became apneic and died.

Autopsy.—The thymus was hemorrhagic and necrotic. The spleen weighed 25 gm. and the liver weighed 360 gm., but no gross changes were observed. The kidneys weighed 30 gm. each and showed numerous petechiae in the pelves. The heart and lungs showed numerous petechiae but no other gross changes. Postmortem blood culture was sterile.

Microscopically, the arterioles and capillaries in almost every organ showed the presence of occlusive material, which was usually homogeneous, refractile, and deeply eosinophilic. Occasionally it appeared somewhat granular or laminated and was noted to contain red blood cells and leucocytes. Similar material was noted beneath the endothelium of many vessels, and occasionally it protruded into the lumen of the vessel. These "hyaline thrombi" were intensely Schiff-positive, homogeneous purple to orange-purple with phosphotungstic acid hematoxylin, and blue with toluidine blue. They were most numerous in the heart (Fig. 21) but were also present in the lungs, the liver sinusoids, and arterioles (Fig. 11), and the renal arterioles and glomerular capillaries (Fig. 27). The presence of this material was often associated with a marked endothelial proliferation, but there was a striking absence of inflammatory reaction. The thymus showed areas of infarction and hemorrhage, and numerous thymic vessels and sinusoids contained material similar to that present in other organs (Fig. 14). Sections from the brain showed no changes.

Incidence of Fibrinoid Following Intravenous Administration of Meningococcal Toxin or Meningococcal Toxin Plus Sodium Polyanethol Sulfonate

Method	No. of Animals	Presence of Fibrinoid				
		Kidney	Spleen	Heart	Liver	Lung
2 cc. of 1:80 toxin × 1	50	4%	10%	12%	16%	16%
2 cc. of 1:80 toxin × 2	27	63%	67%	60%	74%	33%
2 cc. of 1:80 or 1:1000 toxin and 8 mg. sodium polyanethol sulfonate	40	60%	70%	55%	28%	75%

The accompanying Table summarizes the incidence of fibrinoid lesions in rabbits following the administration of meningococcal endotoxin alone or in conjunction with the acidic polymer sodium polyanethol sulfonate. In addition to these regularly occurring lesions, hemorrhage and necrosis of the thymus and adrenals occasionally occur. The latter lesion, however, has been observed only when endotoxin has been given in association with sodium polyanethol sulfonate, and it has been noted in a higher percentage of animals when this polymer is given simultaneously with endotoxin. The occurrence of thymic hemorrhage and necrosis is often associated with severer renal,

splenic, and cardiac lesions, but adrenal hemorrhage has been observed in occasional animals in which other lesions were not found.

COMMENT

A detailed study of the lesions in each of these cases shows vascular alterations characterized by fibrinoid which is frequently associated with areas of hemorrhage and necrosis. The severity of these lesions and the organ or system involved is so variable that in some cases the morphologic diagnosis would depend upon which particular slide one examined. In Case 1, for example, the fibrinoid is confined to the heart and is associated with the typical alterations of acute rheumatic fever (Figs 1 and 5). In the case of scleroderma (Case 3), however, even though the fibrinoid is largely confined to the renal afferent arterioles and glomerular capillaries (Figs. 24 and 28), the manner of its deposition is similar to that observed in lupus erythematosus, in thrombotic thrombocytopenic purpura, or in accelerated hypertension, as well as in scleroderma. In Case 4 the fibrinoid is widely distributed and the lesions produced are similar to those of rheumatic carditis (Fig. 6), lupus erythematosus (Fig. 25), periarteritis nodosa (Figs. 17 and 18), and subacute glomerulonephritis. In each of the other cases the lesions are so widespread and variable that changes characteristic of more than one type may be found.

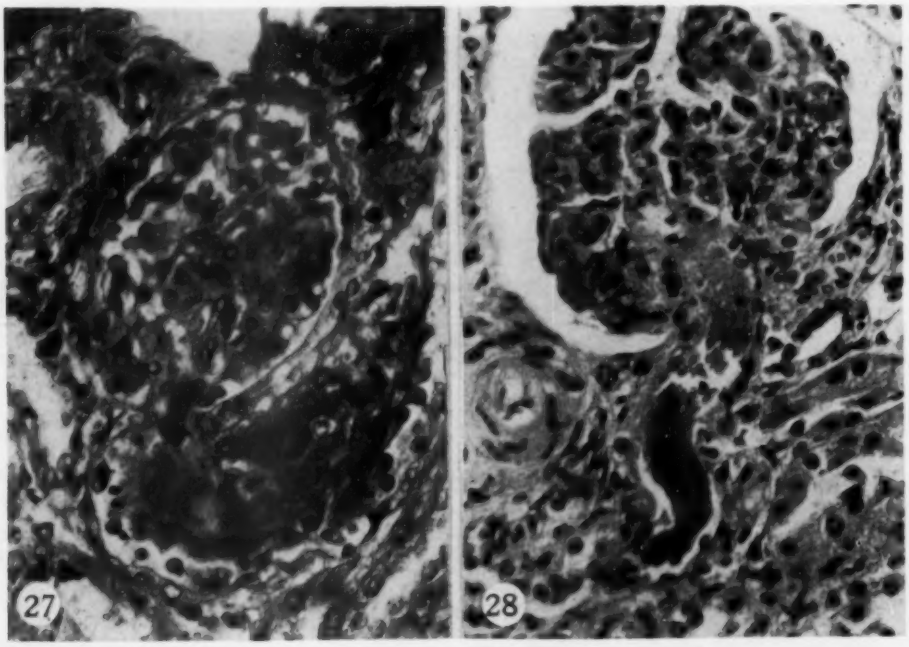


Fig. 27 (Case 6).—Thrombotic thrombocytopenic purpura. Glomerulus showing accumulation of fibrinoid within the lumen and the wall of the afferent arteriole. Hematoxylin and eosin; × 450.

Fig. 28 (Case 3).—Scleroderma. Glomerulus showing fibrinoid lying free within the lumen of the afferent arteriole. Hematoxylin and eosin; × 350.

Thrombotic thrombocytopenic purpura (Figs. 11, 14, 21, and 27) also appears to fall into this group of diseases, as has been suggested by Beigelman.[9] Since the report by Baehr, Klemperer, and Schifrin,[10] it has been assumed by most investigators that the occlusive material in the vessels in this disease is composed of platelets, but

the studies by Gore [11] showed a "prethrombotic" lesion consisting of the presence of a homogeneous acidophilic material beneath the endothelium of the vessels. This material, assumed to be the initial lesion of the disease, gave the usual staining reactions of fibrinoid. Orbison [12] has since shown that primary vascular damage characterized by the presence of fibrinoid and the formation of aneurysms occurs at the arteriocapillary junction in thrombotic thrombocytopenic purpura. In addition to these observations, it should also be emphasized that at the present time no specific tissue stain for platelets exists and the assumption that this material is platelets therefore appears to be unjustified, although it is not improbable that platelets might become enmeshed in the fibrinoid material within the vessels.

The lesions of the generalized Shwartzman phenomenon appear similar, in many respects, to those which have been described in the cases summarized above. This is particularly evident with respect to myocardial (Figs. 3, 7, 8, 20, and 22), valvular (Figs. 2 and 4), and glomerular capillary (Figs. 26 and 30) lesions. The similarity of the renal lesion produced by sodium polyanethol sulfonate to the "wire loops" of lupus erythematosus has been observed previously by Hausman and Dreyfus,[13] who gave large amounts of the polymer intravenously to rabbits. Of interest in this connection is the report by Inderbitzin [14] concerning the production of L. E. cells by the addition of sodium polyanethol sulfonate to suspensions of normal human leucocytes.

Likewise, the deposition of fibrinoid in the spleen, liver (Fig. 9), and lungs (Fig. 13) of the experimental animals is strikingly similar to the human lesions described. In the rabbit, however, the fibrinoid material

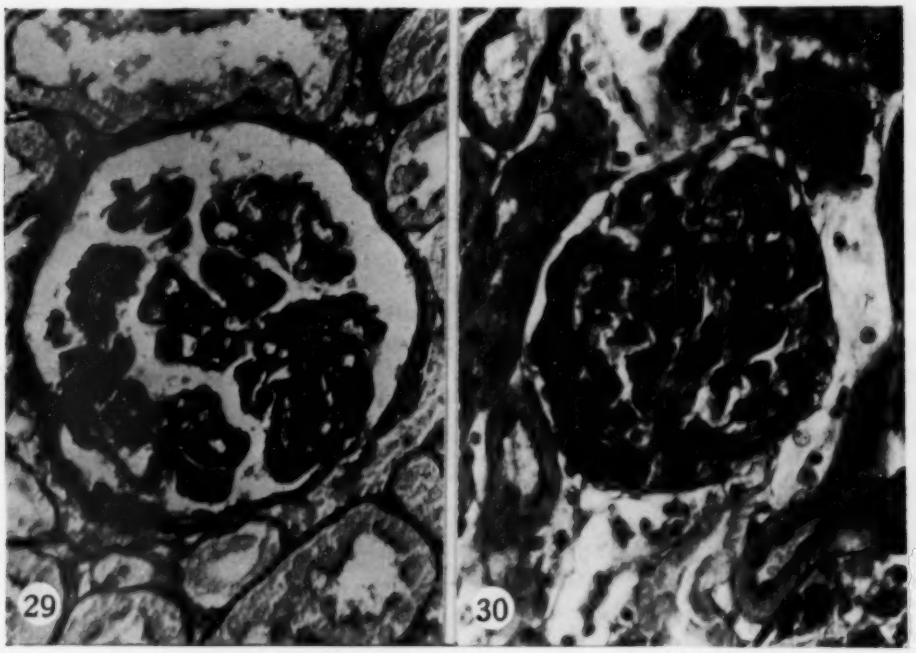


Fig. 29 (Case 5).—Disseminated lupus erythematosus. Glomerulus stained by the periodic acid-Schiff method showing the characteristic "wire loops." × 350.

Fig. 30.—Glomerulus from a rabbit given meningococcal endotoxin and sodium polyanethol sulfonate. Stained by the periodic acid-Schiff method. Note intraluminal fibrinoid and also the "wire loops." × 450.

is oftener associated with gross areas of hemorrhage and necrosis, which are particularly prominent in the spleen and kidneys. This difference is probably related to the rapidity of development of the experimental lesions in contrast to the slower, progressive nature of the human disease processes. One other point of difference is the lack of acute cellular reaction about most fibrinoid lesions involving the arteries of the experimental animals, although this feature is occasionally present (Fig. 16). In the human material which has been presented, however, fibrinoid lesions with and without cellular reaction have been noted in the same case, suggesting that this factor may be of secondary importance. The basic morphologic similarity of the lesions and the identical staining features of the fibrinoid material suggest a similar mode of origin of this material in both the human and the experimental lesions, and it is the additional factors such as the presence or absence of cellular reaction and the major localization of the lesions that permit their separation into individual disease processes. These factors also suggest that, although the derivation of the fibrinoid material may be the same, different etiologic agents may be involved in its production and deposition.

Studies of the generalized Shwartzman phenomenon have indicated that the fibrinoid which accumulates within various organs and tissues is probably derived from the circulating blood,‡ and that fibrinogen may be involved in its formation.§ Its morphologic and tinctorial features are identical with those described by Altshuler and Angevine [5] for fibrinoid occurring in the so-called "collagen diseases." These observations strongly suggest that the basic alteration in these diseases is not a change in collagen or connective tissue. Therefore, it seems more reasonable to refer to them as systemic fibrinoid diseases, at least until more specific details of the various etiologic factors concerned are known.

‡ References 7 and 8.

§ References 8 and 15.

The factors necessary for the production and localization of this material in the human being are not known at present. Experimentally, however, changes in the circulating blood appear to be involved in its production, and structural or functional vascular alterations appear to be important in its localization. These factors and the close similarity between the human and experimental lesions suggest that a more detailed investigation of abnormalities in blood coagulation and serum proteins might provide further information concerning the pathogenesis of these diseases.

SUMMARY AND CONCLUSIONS

Six cases of systemic fibrinoid disease have been presented. The varied lesions occurring in these cases and their morphologic similarity to one another and to the lesions occurring in the generalized Shwartzman phenomenon have been discussed. Factors important in the experimental production and localization of fibrinoid lesions appear to be an alteration in or production of abnormal serum proteins and structural or functional alterations in the endothelium of the cardiovascular system. It is suggested that similar mechanisms may operate in the pathogenesis of the human diseases which have been discussed.

The photographs were made by Mr. Henry Morris.

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Morphology of the Amorphous Intercellular Substance of Hematopoietic Tissues

EVE PERL REAVEN, Ph.D., Chicago

In the present work we have reinvestigated the intercellular components of the lymphatic tissues and bone marrow, employing methods not available to the early workers in the field. From the descriptions of these investigators * the structural frame of hematopoietic tissues was believed to be composed of a syncytial reticular cell network in intimate contact with reticular fibers. In addition, plasma or lymph derived from the vascular or lymphatic vessels was believed to fill the intercellular spaces and to bathe the cells. Little emphasis was placed on the existence of a third component of the intercellular spaces, an amorphous intercellular substance. Heretofore such a substance was described only in pathological conditions of the hematopoietic tissues.† Evidence is presented here to the effect that an amorphous intercellular substance exists in normal, untreated hematopoietic tissues, as well as in the hematopoietic tissues altered through hemorrhage or x-irradiation.

In the description of this substance we utilize as a working definition the concept of "ground substance" advanced by Gersh and Catchpole.[14] According to these authors, the ground substance of frozen-dried tissues is that portion of the intercellular material which is nonfibrillar and optically homogeneous. Under most normal conditions the ground substance is insoluble in dilute alcohols, water, or phosphate buffer at pH 7. With the use of proper controls it may be identified by the periodic acid-Schiff method (PAS).

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From the Departments of Anatomy and Pediatrics, University of Chicago.

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* References 1 to 5.

† References 6 to 13.

MATERIALS AND METHODS

Hemorrhage.—Sixteen young adult (4 to 5 lb.) male and female rabbits were subjected to controlled bleedings. The rabbits were of a snuffle-free strain bred by the National Animal Laboratories. The animals were fed a normal diet of Purina rabbit pellets and had free access to water. Each rabbit was subjected to a single bleeding of 30% of the calculated blood volume[15] by cardiac puncture under sterile conditions. The animals were killed by a blow on the back of the neck at the following intervals after hemorrhage: ½, 1, 3, 5, 8, 12, 18, 24, 36, 48, 72, and 96 hours, and 1, 2, 3, and 4 weeks. Five control rabbits were subjected to sham cardiac punctures and killed either ½ hour or 36 hours later.

X-irradiation.—Two groups of young adult (100 to 150 gm.) male rats were x-irradiated. The animals were fed a diet of Purina Laboratory Chow and had free access to water. Each group, composed of five animals, was exposed to 600 r of x-rays (the L. D. 50/30 days). The x-rays were generated in a 200 kvp machine operating at 15 ma. The x-rays were filtered with 0.25 mm. Cu and 1 mm. Al. Target distance was 70 cm. The animals were placed in individual wire-mesh boxes and were rotated once during the treatment, which lasted 22 minutes. They were killed by a blow on the back of the neck at 1, 8, and 24 hours, and at 8 and 15 days after exposure. Four untreated rats were used as controls.

Histological Methods.—Lymphatic Tissues: Small pieces of spleen and mesenteric lymph node were

removed from the animals and were prepared either by immersion in Zenker-formalin or by freezing and drying. The chemically fixed material was embedded in celloidin, sectioned at 4μ to 8μ, and stained with hematoxylin-eosin-azure II. The frozen-dried material was embedded in paraffin, and the sections were stained by the periodic acid-Schiff procedure for polysaccharide proteins. The method employed the use of alcoholic periodic acid (pH 2) and aqueous leucofuchsin (pH 2.5) and differed from the original method described by Hotchkiss[16] in that several changes of 70% alcohol were used in place of the reducing rinse. Adjacent sections were extracted after denaturation, but prior to staining, in the following solutions: (1) Equal parts of saliva and buffer (M/10 phosphate buffer at pH 7); sections were incubated for one hour at 37 C to remove glycogen. (2) Phosphate buffer (M/10) at pH 7; sections were incubated for one hour at 37 C as a control for (1) above. (3) Hot (60 C) 1:1 methanol-chloroform; sections were extracted overnight to remove soluble glycolipids. Sections not oxidized by periodic acid were stained by the Schiff reagent as a control for naturally occurring insoluble lipids. In addition, sections were extracted prior to denaturation in distilled water, phosphate buffer (pH 7), and dilute alcohol in order to observe the solubility of the intercellular components in these fluids.

Bone Marrow: Small pieces of bone marrow from the proximal end of the femur were removed from each animal and prepared either by immersion in Zenker-formalin or by freezing and drying. The chemically fixed material was embedded in celloidin, sectioned at 8μ, and stained with hematoxylin-eosin-azure II.

With the frozen-dried material two staining procedures were used, the periodic acid-Schiff method (PAS) and the zinc acetate-spirit blue method. In the first method, sections were cut at 4μ and 10μ, deparaffinized with xylene, denatured overnight in 95% alcohol, and stained for polysaccharide proteins as described under the lymphatic tissues. Saliva, M/10 phosphate buffer at pH 7, and methanol-chloroform-extracted sections were used as controls. Components of the amorphous intercellular substance were stained pale pink to red; plasma and lymph proteins showed a trace of pink color.

The second method of staining was based on the experience of Cohn and his collaborators[17] that a maximal amount of plasma proteins could be precipitated in the test tube if a combination of factors, known to favor protein precipitation, were used in a single solution. They suggested the use of a bivalent ion such as zinc in a nonpolar solvent which was buffered at pH 4.8, the isoelectric point of plasma albumin. They found that if the solution was precooled to —5 C the precipitated proteins were not denatured and could be fractionally redissolved. The zinc acetate solution used in this study was important in retaining proteins presumably already precipitated by the freezing and drying of the tissues, thus differing in its primary function from the precipitating solution used by Cohn.

Of the various solutions tested in the course of this work for the preservation and staining of intercellular substance, the following seemed to be the most effective: 10 gm. of zinc acetate dissolved in 25 cc. of boiling water to which 125 cc. of 95% alcohol was added. The solution was acidified with enough HCl to give a reading of pH 2.7 at 25 C in an instrument using glass and calomel half-cells. The pH was critical to within 0.1 of a unit. The solution was then made up to 250 cc. with distilled water and cooled to —5 C.

The procedure was as follows: Thin (4μ) sections were placed on unalbuminized coverslips with gentle finger pressure. The paraffin was removed from the sections by petroleum ether which had been recently redistilled over sodium to remove water. The coverslips were then cautiously flamed to remove the ether and were immediately immersed in the cooled zinc acetate solution, where they remained from 12 to 24 hours. In order to retain the now insoluble components for subsequent staining, the sections were transferred for 12 hours to a solution of 5% phosphotungstic acid in the —5 C zinc acetate solution described above. The sections were then rinsed in cooled absolute alcohol and stained for two minutes in a 1% alcoholic solution of spirit blue at room temperature. The intercellular substance was stained a deep blue color, readily distinguished in most sites from the various shades of blue of the cells. Some sections were extracted at the stage prior to immersion in the zinc acetate solution with distilled water or with various solutions of alcohol (5% to 95%) at —5 C and at room temperature. This was done to get an indication of solubility of the amorphous intercellular substance.

RESULTS AND COMMENT

Lymphatic Tissues.—The amorphous intercellular substance of the normal lymphatic tissues usually stained a pale pink with the PAS procedure. At high magnifications the substance was not homogeneous. It appeared to consist of a finely reticulated, close-meshed "net" of relatively uniform dimensions, which completely surrounded the lymphocytes. This "net" arose during the initial freezing of the tissue specimens in isopentane, through the separation of water in crystalline form from the protein component

of the insoluble ground substance. Although the reticular fibers were also stained pink, they were frequently thicker than the apparent strands of the ground substance-net and were sharply delimited. For the most part, the distribution of the reticular fibers is not so extensive or uniform as to be confused with the net of the ground substance which extends between all cells. The plasma protein precipitated within the confines of the vessels and sinusoids appeared as a relatively coarse net compared with the amorphous intercellular substance in the surrounding areas. Furthermore, the walls of this intravascular "net" were generally swollen and diffusely outlined after extraction in dilute alcohols and water; because of this the intravascular plasma proteins were considered to be partially soluble in the test fluids. Fibers and the ground substance were, for the most part, insoluble. The exceptions to this description will be noted in detail later.

The cytoplasm of the lymphocytes and of the majority of undifferentiated reticular cells was not stained by the PAS method. The cytoplasm of the macrophages was usually pale pink and sometimes contained debris of a purplish hue. The exact relationship between the cells of an area and the appearance of the amorphous intercellular substance in the same area was defined by comparison with adjacent PAS sections which were counterstained with toluidine blue.

With one exception, the distribution, staining, and form of the amorphous intercellular substance was quite consistent throughout the major areas of the normal lymphatic tissues. An insoluble finely reticulated net was visible between all cells of the white pulp of the spleen and of the nodules of the mesenteric lymph node. The intensity of the PAS stain varied little. Only the ground substance of the marginal zones of the splenic nodules and of the "resting" or "inactive" centers of the lymph nodules seemed to carry a deeper stain than surrounding areas, but this difference was not striking. However, the amorphous intercellular substance of the extranodular lymphatic areas of the lymph node was soluble in the test fluids if the latter were used prior to alcoholic denaturation. Furthermore, the amorphous substance of this area was precipitated in a coarse net. In both these respects the intercellular substance resembled the precipitated intravascular proteins.

After hemorrhage or x-irradiation, notable changes in the stainability and distribution of the amorphous intercellular substance took place in the lymphatic organs. Many of these changes accompanied the death, removal, or migration of large numbers of cells from these organs. In effect, the ground substance appeared to fill in spaces vacated by cells, and in many early stages of this change the stainability of the ground substance was greatly increased.

After hemorrhage, the ground substance in all areas of the white pulp of the spleen stained redder than in the controls, especially the marginal zones of the nodules and the diffuse lymphatic tissues of the periarterial sheaths. At the same time, the outlines of the marginal zones were diffuse and seemed to merge with the red pulp. Lymphocytes of any given size were not so rigidly confined to specific areas as they were in the control tissues of these animals, and, in general, the cells were not so tightly packed. These changes in the cellular pattern were apparent at three hours after hemorrhage and continued to be prominent in the white pulp during the first week. In most areas, staining changes in the ground substance took place concomitantly with changes in the density and arrangement of cells. In the lymph node there was no apparent change in the total number of nodules or in the size of the nodules after acute hemorrhage. However, as early as three hours after hemorrhage a large number of nodules showed internal alterations such as the appearance of very large and actively mitotic lymphopoietic centers. In addition, the coronas appeared thinner than in the nodules of the control animals. The amorphous intercellular substance in the corresponding nodules stained a brilliant red with the PAS procedure. By 18 and 24 hours, many of the

nodules contained large inactive centers containing lymphocytes of various sizes, many macrophages, and a few reticular cells. The ground substance stained strikingly red at these stages. Gradually, as the nodules returned to their normal appearance (between one and three weeks), the intensity of the ground substance stain decreased.

After irradiation, the cellular events were quite uniform. The massive death and removal of cells was accompanied by partial destruction of the original architecture of the organs involved.[18] A red-staining ground substance appeared to occupy the cell-free areas. In the spleen, ground substance changes were apparent as early as one hour after irradiation and were most striking after one day. By eight days the changes were still marked. In the lymph node, by 24 hours no nodular structure had remained. Yet, between the reticular cells intensely bright regions of color identified the ground substance of the former nodules. In this organ, however, the color of the ground substance stain was greatly decreased by eight days.

In no instance was the solubility of the amorphous intercellular substance in the test fluids altered as a result of the experimental procedure.

In general, alterations in the cell pattern of the organs studied was reflected in a brightly stained ground substance, which appeared to fill out the spaces recently vacated by cells. It appears as if the ground substance changes were concomitant with, or secondary to, the cellular changes.

The nature of the ground substances deposited over this increased area may be roughly defined through the following assumption. The degree of color attained by the PAS procedure depends on the number of reactive carbonyl groups which are available for combination with the dye. An increase in color of the stain could be accounted for by an increase in available groups through the deposition of more compounds with the reactive groups of a depolymerization or disaggregation of the existing compounds. According to Gersh and Catchpole[14] the altered compound, if sufficiently depolymerized, should be more easily soluble than its more highly polymerized form. In the present experiment, the solubility of the ground substance did not change according to the methods we employed. It may then be assumed that the increase in stain of the amorphous intercellular substance was the result of newly formed and deposited ground substance, or a slight degree of depolymerization, or both.

Finally, there is some reason to believe that with the freezing methods currently employed the rate of crystal growth in heavily water-laden tissues may result in unusually large ice-crystal artifact. In the lymphatic tissues, the size of the apparent "net" or ice-crystal artifact was consistently fine in the white pulp of the spleen and in the nodular areas of the lymph node. The size of the "net" was consistently coarse in the extranodular areas of the lymph node and in this way resembled the coarse net of the precipitated intravascular proteins. The similarity of the size of the nets suggests that the soluble amorphous intercellular substance of the extranodular areas of the lymph node may approach the consistency of such fluid tissues as blood and lymph.

Bone Marrow.—The amorphous intercellular substance of normal bone marrow was found to be dissolved in dilute alcohols, in buffer, and in water. For this reason it could not be demonstrated by the PAS method. When fixed and stained by the zinc acetate-spirit blue method, however, amorphous intercellular substance was present around the bone marrow cells as a blue-staining material with a uniformly delicate net-like structure. The "net" structure is the form of the protein residue which remains after sublimation of water from the ice crystals which form during the freezing process.

In considering the amorphous intercellular substance of bone marrow, it became apparent that one must expand the general concept of ground substance to include a highly soluble fraction which exists also in normal, untreated tissues. The value of the zinc

acetate procedure in this study is that it preserves large amounts of such soluble intercellular material. The use of the method is limited, in that the quantity of the preserved amorphous material is occasionally so great and deeply stained that small, or even large, variations in amount escape detection. Hence, due to the lack of sensitivity and of specificity of the procedure, it was employed only to detect the presence of intercellular material which was unstainable by other methods.

During the first few hours after acute hemorrhage, no intercellular substance could be demonstrated by the PAS method, although, like the ground substance of normal marrow, it could be stained by the zinc acetate-spirit blue method. By eight hours, however, faint pink traces of color appeared between groups of cells, and by 24 and 36 hours the quantity of PAS-stainable material had increased significantly. At this time and during the following 12 to 24 hours, there were relatively few cells in the marrow and the depleted areas were now filled with an amorphous substance which stained red with the PAS method. In the 72-hour and 96-hour specimens, despite the fact that the marrow was again filled with increased numbers of cells, the intercellular material remained stainable by the PAS method. In the one-week specimen, however, this was no longer true.

In the irradiated groups, by eight hours after exposure many cell-poor areas were noted in the bone marrow in which the PAS-stainable intercellular substance was prominent. During the following week the quantity of the amorphous ground substance continued to increase at the expense of the cells. At the same time the stainability of the ground substance was enormously increased. This stage corresponded morphologically with previous descriptions of "gelatinous" marrow.‡

It seems that the amorphous intercellular substance of the normal bone marrow differs from the intercellular substance in the experimental marrow only in the actual amount of the ground substance present. In the normal bone marrow, ground substance is not visible with the usual stains and with the PAS method, since such a large proportion of the ground substance is soluble in the water solutions used in the methods. With the zinc acetate-spirit blue method, however, an amorphous material was identified between the marrow cells. In adjacent sections which were extracted for one-half hour with dilute alcohols (50% and lower), M/10 phosphate buffer at pH 7, or distilled water, before denaturation of proteins in the zinc acetate solution, this amorphous substance was no longer stainable. Presumably it had been washed out in the test fluids. In identical extractions involving sections of bone marrow from the experimental animals, the ground substance proved to be equally soluble. The fact that the ground substance of this tissue stained with the PAS method is explainable on the basis that the increase of intercellular substance was so considerable that a portion of the substance could still be available for staining, even though the major part had been dissolved in the fixing and staining fluids.

The steady increase of staining of the amorphous intercellular substance with the PAS method following hemorrhage or x-irradiation can also be explained on the basis that new ground substance is being formed and deposited, and therefore reactive groups are increasingly available for combination with the dye.

SUMMARY

An insoluble amorphous intercellular substance is described in sections of control rabbits and rats in nodules of the mesenteric lymph node and in all areas of the white pulp of the spleen. A soluble component of the amorphous intercellular substance is described in the diffuse extranodular lymphatic tissue of the lymph node.

The chief effects of acute hemorrhage or x-irradiation on the amorphous intercellular substance in the tissues studied is observed

‡ References 10 to 13.

by use of the periodic acid-Schiff (PAS) method. There is a marked increase in stainability and in distribution of the ground substance. The changes observed in the stainability of the ground substance after x-irradiation are often more pronounced and more prolonged than after acute hemorrhage.

An amorphous intercellular substance is described in the bone marrow of normal, untreated rabbits and rats and in the bone marrow of animals stimulated through severe hemorrhage or x-irradiation. The demonstration of this intercellular substance is possible in frozen-dried tissues through the use of the periodic acid-Schiff technique and a new method, the zinc acetate-spirit blue method, which is used to precipitate and stain intercellular material not stainable by other methods.

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Hypertension and Coronary Arteriosclerosis

A Comparative Study of the Weights of Kidneys in Patients with Acute Myocardial Infarction, Systemic Hypertension, Carcinoma, and Nonrenal Disease

KYU TAIK LEE, M.D.
and
WILBUR A. THOMAS, M.D., St. Louis

The relationship between coronary arteriosclerosis and increased arterial pressure has been a subject for discussion and investigation for a long time. It is well established that hypertension is a common concomitant of coronary arteriosclerosis in both men and women. Most investigators have concluded that hypertension is an important etiological factor in the development of coronary arteriosclerosis,* but there have been some dissenting opinions. Master and his associates † made an extensive study of the systemic blood pressures in 600 patients with a clinical diagnosis of coronary occlusion and compared them with the blood pressures in a "control" group consisting of 74,000 working men and women. In men, these workers found systemic hypertension to be equally common in the two groups ("coronary occlusion" and "control"), suggesting to them that hypertension is not a significant etiological factor in the production of coronary occlusion in men. In women they did find a significant correlation between systemic hypertension and coronary occlusion and decided that, in women, hypertension is probably a factor of etiological importance. In a similar, very recent, clinical study, Sigler [14] concluded that hypertension does not appear to have any definite causal or prognostic relationship to coronary occlusion in either men or women.

Further dissent has come from experimental studies. Goldblatt [15] could not demonstrate significant degrees of arteriosclerosis in dogs that had had systemic hypertension for as long as seven years without accompanying renal excretory functional disturbance.

In a recent study in our own laboratory [10] of the relationship between pulmonary arteriosclerosis and pulmonary hypertension in patients with congenital heart disease, we found pulmonary arteriosclerosis to be equally common and equally extensive in patients with and without pulmonary arterial hypertension. The severity of lesions was somewhat greater in the patients with pulmonary hypertension.

Thus it is apparent that the relationship between arterial hypertension and arteriosclerosis is not entirely clear. We have looked for an objective method, based on autopsy data, for evaluating the role of systemic hypertension in coronary arteriosclerosis.

It is well established that most patients who had systemic hypertension during life will have at autopsy significant degrees of arteriolar nephrosclerosis and that arteriolar nephrosclerosis is associated with a decrease in the size and weight of the kidney. An objective method for the evaluation of the role of hypertension in coronary arteriosclerosis might be based on these two facts.

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Department of Pathology, Washington University, St. Louis.

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Dr. Lee is Research Fellow in Pathology and Dr. Thomas is Assistant Professor in Pathology, of the Department of Pathology, Washington University.

* References 1 through 10.

† References 11 through 13.

The method that we have used consists of a comparison of the weights of kidneys in a large number of patients who had died of acute myocardial infarction with corresponding weights obtained from several comparative groups. The patients with acute myocardial infarction constitute a group with definite evidence of advanced coronary arteriosclerosis.

Three other groups of patients were chosen for comparison: 1. In order to establish the weights of the kidneys in patients dying with a principal diagnosis of advanced hypertension associated with arteriolar nephrosclerosis, a group was chosen consisting only of patients with such a diagnosis. (Patients with glomerulonephritis or pyelonephritis were not included.) 2. In order to establish the weight of the "normal" kidney, a group of patients was chosen having no anatomic evidence of significant degrees of renal disease. Almost all older patients in our autopsy series had at least slight arteriolar nephrosclerosis. Thus it was impossible to secure a group that can be considered entirely "normal," but it is as nearly "normal" as it was possible to obtain. 3. In order to establish the weight of the kidney in a cross section of the population without regard to the presence or absence of hypertension, a group of patients was chosen who had died of carcinoma (excluding renal, adrenal, and pituitary tumors). It seems reasonable to assume that for our purpose such a group represents a satisfactory sample of the population, since there is no reason to believe that carcinomas unassociated with the production of hormones are in any way related to hypertension.

MATERIAL AND METHODS

Four groups consisting of consecutive series of patients from the autopsy files of the department of pathology of Washington University were established in the following manner: 1. "Myocardial Infarction" Group: This group was composed of 300 patients (193 men, 107 women) who had a principal anatomical diagnosis of acute myocardial infarction. 2. "Hypertension" Group: This group was composed of 90 patients (58 men, 32 women) over 30 years of age who had a principal diagnosis of systemic hypertension. 3. "Carcinoma" Group: This group was composed of 100 patients (58 men, 42 women) over 30 years of age who had a principal diagnosis of carcinoma. 4. "Control" Group: This group consisted of 100 patients (62 men, 38 women) of comparable age, with no anatomical evidence of significant renal disease.

From the autopsy protocols we obtained the following types of data on each of the patients in these four groups: sex, age, and the weights of the body, kidneys, heart, and lungs. The autopsies had been performed and the protocols written by the house staff of the department of pathology. According to the rules of the department, loose fat and other accessory tissue are removed from organs before they are weighed. Techniques of weighing and preparing organs for weighing no doubt varied and constitute a source for error. However, such variation applies to all groups, and the resulting inaccuracies should not be any greater in one group than in any of the others.

RESULTS

The results are summarized in Tables 1 and 2. The average weight of the kidneys was greatest in the control group (375 gm.), and least in the hypertension group (278 gm.). The average weights of the kidneys in the myocardial infarction group (332 gm.) and the carcinoma group (332 gm.) were equal, and both fell between the two extremes (control and hypertension groups).

In men the average weight of the kidneys was slightly greater in the myocardial in-

TABLE 1.—*Average Weights of the Kidneys in Four Groups of Patients*

	Myocardial Infarction	Carcinoma	Hypertension	Controls
Total number of cases	300	100	90	100
Male	193	58	58	62
Female	107	42	32	38
Male	355 gm.*	347 gm.	284 gm.	388 gm.
Female	290 gm.	313 gm.	268 gm.	356 gm.
Male and female	332 gm.	332 gm.	278 gm.	375 gm.

* The averages are of the combined weight of both kidneys in each case.

TABLE 2.—*Average Ages and Average Weights of the Bodies, Hearts, and Lungs in Four Groups of Patients*

	Sex	Myocardial Infarction	Carcinoma	Hypertension	Controls
Heart	M	542 gm.	382 gm.	625 gm.	402 gm.*
	F	468 gm.	309 gm.	560 gm.	367 gm.
	M & F	515 gm.	349 gm.	602 gm.	389 gm.
Lungs	M	1,343 gm.	1,303 gm.	1,455 gm.	1,425 gm.
	F	963 gm.	1,039 gm.	1,158 gm.	947 gm.
	M & F	1,203 gm.	1,203 gm.	1,349 gm.	1,244 gm.
Body weight	M	69 kg.	61 kg.	67 kg.	64 kg.
	F	61 kg.	56 kg.	59 kg.	61 kg.
	M & F	65 kg.	59 kg.	64 kg.	63 kg.
Age	M	62 yr.	60 yr.	50 yr.	61 yr.
	F	65 yr.	58 yr.	49 yr.	61 yr.
	M & F	63 yr.	59 yr.	49 yr.	61 yr.

* Includes some patients with valvular heart disease, since this group was chosen only on the basis of absence of "significant" renal disease.

farction group (355 gm.) than in the carcinoma group (347 gm.). In women the kidneys were slightly smaller in the myocardial infarction group (290 gm.) than in the carcinoma group (313 gm.).

The average weight of the hearts was greatest in the hypertension group (602 gm.) and least in the carcinoma group (349 gm.). The average weight of the hearts in the myocardial infarction group (515 gm.) was considerably less than that in the hypertension group, but significantly greater than that in either the carcinoma group or the control group (389 gm.). (The average weight of the hearts in the control group cannot be considered normal because it includes some patients with valvular heart disease, since this group was chosen only on the basis of absence of "significant" renal disease.)

The average weights of the lungs and average body weights were similar in all of the four groups. The average age was similar in three of the groups (myocardial infarction, 63 years; control, 61 years, and carcinoma, 59 years), but lower in the hypertension group (49 years).

COMMENT

The marked difference between the average weight of the kidneys in the hypertension group and that in the control group confirms the basic premise that the weight of the kidney is decreased in patients with systemic hypertension. The differences were similar in both sexes (the average weight of the kidneys in the hypertension group was 27% less in men than the corresponding weight in the control group, and 24% less in women), and both differences are statistically highly significant (p less than 0.01).

The difference between the average weight of the kidneys in the myocardial infarction group and that in the control group is highly significant (p less than 0.01 for both men and women) and is consistent with the generally accepted idea that hypertension is a common concomitant of advanced coronary arteriosclerosis. The average weight of the kidneys in the myocardial infarction group was 9% less in men than the corresponding weight in the control group, and 18% less in women. The fact that the difference is twice as great in women as in men supports clinical observations ‡ that indicate hypertension is more commonly associated with myocardial infarction in women than in men.

The most important observation in the present study is the absence in men of any significant difference between the average weight of the kidneys in the myocardial infarction group (355 gm.) and that in the carcinoma group (347 gm.). If our basic assumptions are valid, these figures signify that hypertension is no more important in

‡ References 1 through 10, 13, and 14.

men in the etiology of coronary arteriosclerosis than it is in the etiology of carcinoma. Since there is no reason to assume that hypertension is related in any way to carcinoma (unassociated with the production of hormones), these data support the idea that hypertension is not important in the pathogenesis of coronary arteriosclerosis in men.

In women the difference between the average weight of the kidneys in the myocardial infarction group (290 gm.) and that in the carcinoma group (313 gm.) is slight (7%) and is not statistically significant ($p = 0.1$). Thus our data fail to provide definite support for the idea that hypertension is an etiological factor in coronary arteriosclerosis in women, but they are compatible with this concept.

In contrast to the weights of the kidneys, the average weight of the hearts in the myocardial infarction group was significantly greater than that in either the carcinoma group or the control group. However, there are several objections to accepting the weight of the heart as a guide to the presence of hypertension in patients with acute myocardial infarction. 1. The weight of the heart is altered, at least to some extent, by myocardial edema, hemorrhage, and the inflammatory reaction associated with the infarct. 2. Some degree of myocardial hypertrophy may occur in patients with advanced coronary sclerosis simply as a result of dilatation of the heart and overstretching of muscle associated with myocardial ischemia. Both of these objections apply only to the heart and not to the kidneys.

If generalized edema had been present to a significantly greater extent in one group than in the others, the weight of the kidneys might have been disproportionately altered. However, the similarity of the average body weights and of the average weights of the lungs in the four groups suggests that, terminally, generalized edema was not significantly greater in one group than in the others. It must be emphasized that these data are representative only of the terminal state and cannot be interpreted to imply that edema is of similar importance in all groups throughout the clinical course.

The weights of the kidneys and other organs are related in a general way to body size. The similarity of the averages of the body weights of the four groups indicates that factors related to body weight were of no importance in this study.

The average ages were similar in three of the groups, but were lowest in the hypertension group. The fact that the hypertension group was younger does not affect any of our conclusions, because it is reasonable to assume that if the members of this group had lived as long as the members of the other groups, the average weight of the kidneys in the hypertension group would have been even less, and differences already observed would simply have been accentuated.

SUMMARY

In an attempt to obtain objective evidence pertaining to the relationship between systemic hypertension and coronary arteriosclerosis, a comparative study of the weights of kidneys has been made in four large groups of patients dying from (1) acute myocardial infarction (300 patients), (2) systemic hypertension (90 patients), (3) carcinoma (100 patients), and (4) nonrenal disease (100 patients).

The study is based on the facts that (1) arteriolar nephrosclerosis is a common concomitant of systemic hypertension and (2) arteriolar nephrosclerosis results, in general, in a decrease in the size of kidneys.

In men, the average weights of the kidneys were similar in patients with myocardial infarction (355 gm.) and patients with carcinoma (347 gm.), this observation supporting the idea that hypertension is not important in the pathogenesis of coronary arteriosclerosis in men.

In women, the average weight of the kidneys was slightly less in patients with myocardial infarction (290 gm.) than in patients with carcinoma (313 gm.), but the difference is not statistically significant (p=0.1). Thus

these data fail to provide definite support for the concept that hypertension is an etiological factor of importance in coronary arteriosclerosis in women.

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Time Trend of Hyperlipoproteinemia After Radiation Injury

NORMAN WEINER, M.D.
HARRY G. ALBAUM, Ph.D.
LAWRENCE J. MILCH, Ph.D.
and
THE CARDIOVASCULAR RESEARCH GROUP,
Randolph Field, Texas

Significant increases in plasma cholesterol, phospholipids, and low-density lipoproteins following x-irradiation to the hindlimbs of rabbits have been reported from this laboratory.[1] The increases were found to be proportional to the magnitude of the radiation dosage, with the largest rise occurring between levels of 20,000 and 25,000 roentgens. The x-ray dose and plasma alterations correlated well with the histologic changes occurring in the hindlimb muscle itself. These data extended previous findings from this laboratory that local injury of any type can produce plasma lipid elevations.[2] One interesting difference which has been observed is that the phospholipid-cholesterol (P/C) ratio declines after radiation injury but remains normal after other types of trauma, lipid phosphorus and cholesterol levels rising proportionately in the latter groups.[2]

Radiation injury is a form of trauma characterized by a latent period following radiation exposure.[3] Visible damage to parenchymal cells and supporting tissues proportional to the level of dosage occurs subsequently. Inflammatory response is elicited, and if the radiation damage is severe, thrombosis and blood vessel degeneration may occur. Regeneration or fibrosis ultimately occurs, depending, again, on the type of tissue and the radiation dosage. The whole process of radiation injury and repair may be complicated by infection, to which the injured tissues are especially susceptible.[3]

Because of these complex events following local radiation, it was thought that an investigation of plasma lipid changes at repeated intervals after trauma and their correlation with local tissue injury, as determined chemically and histologically, would prove of interest.

METHODS

A total of 176 male albino rabbits of the New Zealand strain, each weighing between 2.5 and 3.0 kg., were divided equally into 11 group-pairs. Each group-pair was subdivided into two equal groups, A and B, Groups A representing the experimental groups, and Groups B representing the controls. Groups A were subjected to local x-radiation of 20,000 r, delivered by a Picker x-ray machine at 260 kvp, 18 ma., inherent filtration of 0.25 mm. Cu, at an entrance dose rate of 570 r per minute and a target distance of 29 cm. The radiation was directed to the distal portion of the right hindlimb. Stray radiation to the rest of the body was eliminated by inclosing the animal in a hollow tube of one-half inch lead, with a small hole at one end through which the limb of the animal extended. The x-ray tube was directed at this limb, and the hollow lead shield was further extended around the limb-x-ray tube system to absorb stray radiation. The experimental setup is shown in Figure 1. To insure reproducible radiation each animal was carefully tied in the tube, and the animal's position rechecked after the radiation period. Following the radiation, Groups

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From the Department of Pharmacology-Biochemistry, School of Aviation Medicine, U. S. A. F., Randolph Air Force Base, Randolph Field, Texas (Dr. Weiner, Dr. Milch, and the Cardiovascular Research Group: A/1C Lesly G. Robinson, A/1C Johnnie F. Trickel, A/2C Richard Berthiaume, A/2C Beulah L. Croye, and A/2C Gordon E. Shults); from the Department of Biology, Brooklyn College, Brooklyn, N. Y. (Dr. Albaum).

A and B were returned to the animal room, placed in individual cages, and maintained on Purina rabbit chow and water ad libitum. All animals were carefully weighed and examined every other day, any abnormality in general condition or gross alterations in the injured limbs of Groups A being noted. No treatment was administered to the injured limbs, even when infection supervened.

Group-pairs of rabbits were killed at 1, 3, 5, 7, 9, 11, 13, 15, 19, 21, and 47 days postexposure. The order of irradiation of the group-pairs was randomized to eliminate the effect of any uncontrollable temporal factors.

Prior to killing, 30 ml. of blood was obtained by cardiac puncture, by use of a lightly heparinized syringe. The animal was then anesthetized with intravenous thiopental (Pentothal) sodium, and the tibialis anticus muscle of the right hindlimb removed. The proximal half of the muscle was analyzed for adenosinetriphosphate (ATP),[4] and its per cent dry weight determined by heat dehydration. The remaining half of the muscle was immersed in formalin for subsequent histological examination. Representative specimens of other organs were also removed for this purpose.

A small portion of the blood removed was used for hematocrit determination. The remainder was centrifuged at low speed, and the plasma removed for analysis of total cholesterol,[5] phospholipid,[6] and the various classes of lipoproteins.*

To confirm values at some of the more critical points, repeat experiments were performed at 5, 7, 11, and 13 days postexposure, using a total of 64 additional animals. These data correlated closely with the previous work and were, therefore, combined with it.

The results of biochemical analyses, shown in the graphs, are expressed as the difference between experimental and control means, ± 2 standard deviations of the mean difference.[9] Significant differences from control values, discussed below, have been determined by the "Student" t-test.[9] Significant changes in the time curves have been determined by the multiple range test.[10] Unless otherwise noted, only those values found highly significant ($P<0.01$) are discussed. The average value of each biochemical determination, utilizing all control animals, has been inserted in the graphs, so that approximate absolute levels may be computed by the reader, if desired.

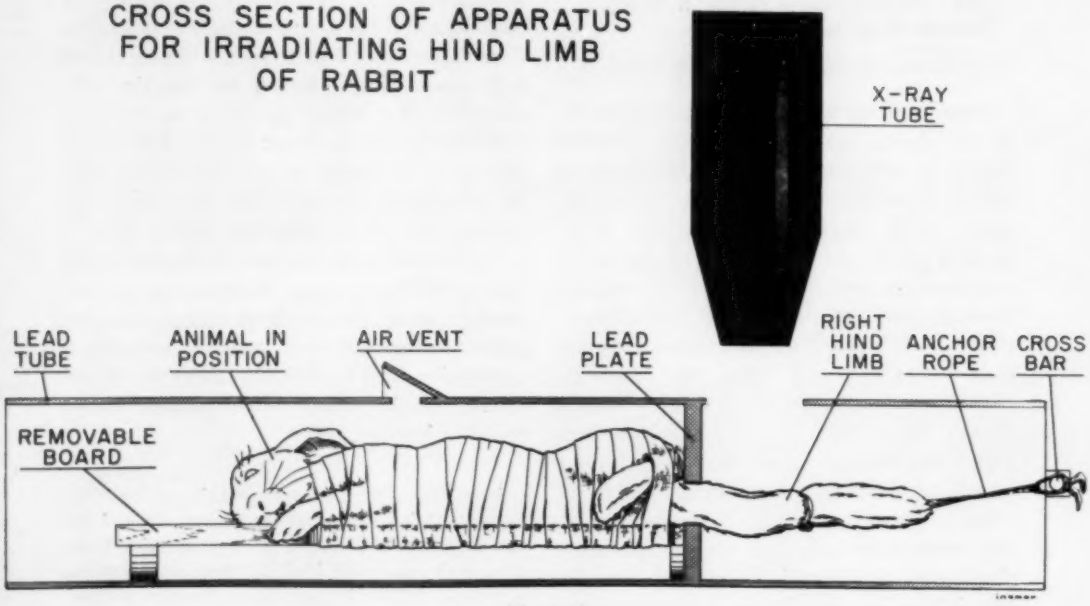

Figure 1

RESULTS

Gross and Microscopic Pathology.—The irradiated limbs underwent a reproducible series of changes. No gross alterations occurred during the first three days postirradiation. Thereafter, swelling appeared and progressively increased over the next 10 days. Epilation of the irradiated tissue began at the 6th day and continued throughout the next week until, at the 13th day, the

* References 7 and 8.

epilation was complete. At the 7th day, the visible limb tissues became hyperemic and hemorrhagic, and by the 10th day a serosanguineous fluid transuded from the injured site. About the 15th day, the transudation subsided and the tissues became brittle, dry, and necrotic, presenting the picture of dry gangrene. This gangrenous phase was followed by sloughing of the subcutaneous and muscle tissues until about the fifth week when, in many cases, only denuded bone remained. Subsequently some limbs underwent spontaneous amputation. No attempt was made to treat the occasional infection present at the stump.

The microscopic appearance of the muscle approximately reflected the changes which occurred in the more superficial tissues. Little alteration occurred over the first three days, except for some equivocal increase in inflammatory cells between the fibers. The inflammatory cell infiltration became definite at five days, and edema and some loss of muscle structure was noted. At the end of the first week blood vessel degeneration was observed and an occasional arteriole thrombus was seen. Polymorphonuclear infiltration became marked around the 9th day and persisted for several days. Resorption of the inflammation with organization began about the 13th day. The degeneration of muscle fibers with loss of tinctorial properties was complete at the end of the third week, giving the entire muscle area the appearance of an infarct. Representative histological sections are shown in Figure 2.

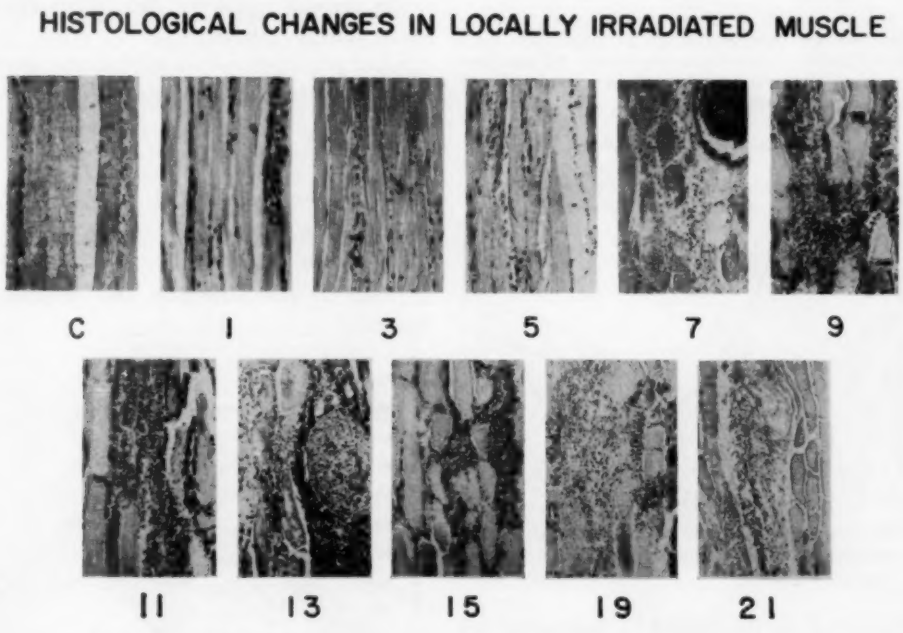

Figure 2

ATP: Muscle ATP analyses were performed in order to have a quantitative measure of the extent of local radiation injury. The results are shown in Figure 3. The ATP values are well correlated with what might be expected from the microscopic appearance of the muscle. The dry weight per cent (Figure 4) falls over the first 11 days, cor-

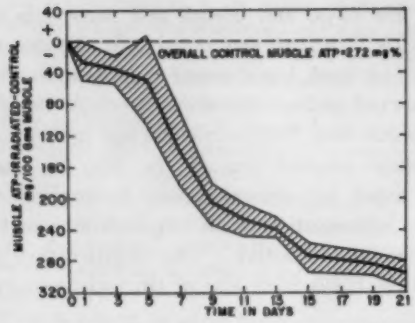

Fig. 3.—Muscle ATP changes following x-radiation. Results are expressed as departuré from control levels. Shaded area represents ±2 standard deviations of the mean difference between irradiated and control groups.

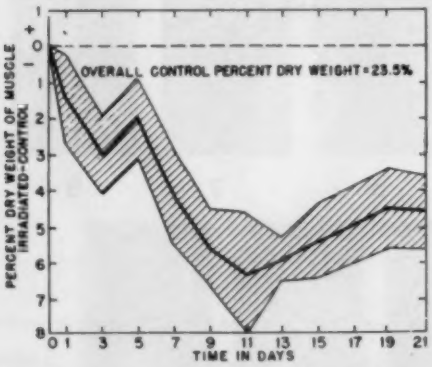

Fig. 4.—Changes in per cent dry weight of muscle from control levels following x-radiation ±2 standard deviations.

responding to the period of increasing inflammation and edema. Thenceforth the per cent dry weight increases progressively, reflecting the subsidence of inflammation and edema and the increasing organization and fibrosis.

Cholesterol and Lipid Phosphorus: Plasma cholesterol concentrations become significantly elevated at 5 days, rise further to a maximum of 130 mg. per 100 cc. at 19 days, and fall significantly to a mean value of 62 mg. per 100 cc. by the 47th day. This final level is still probably significantly elevated as compared with that of its control group ($P<0.05$). The considerable variation in individual values is reflected in the irregular rise of the curve from the 5th to the 21st day, as well as in the large variances calculated. That the reversals in cholesterol concentrations are real for these groups of animals is supported by similar experimentally observed declines in lipid phosphorus and lipoprotein levels at these stages, the three values ordinarily correlating in a general way. However, in none of these instances were the transient declines significant beyond the 0.1 level (t-test). Possibly with much larger groups of animals these fluctuations might become significant, but at present no such claim is justified.

Lipid phosphorus does not become significantly elevated until the seventh day post-irradiation. It follows a curve similar to that of cholesterol but fails to control levels by the 47th day.

The P/C ratio becomes significantly depressed at five days, and falls further over the next two weeks. This low level persists at least to the 47th day, and apparently beyond.

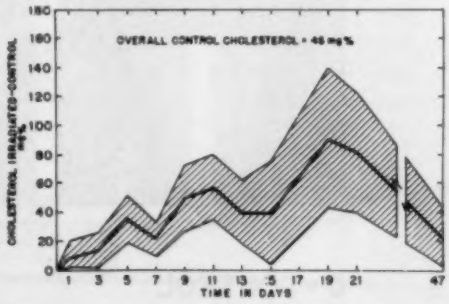

Fig. 5.—Plasma cholesterol changes from control levels after x-radiation ±2 standard deviations.

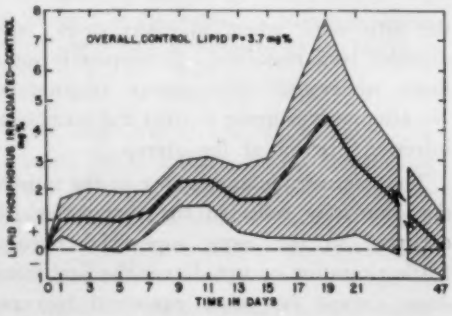

Fig. 6.—Plasma lipid phosphorus departures from control levels after x-radiation ±2 standard deviations.

Lipoproteins: The S_f 20-400 class of lipoproteins becomes elevated by the first day postirradiation. Subsequent to this, the levels undergo considerable fluctuation but achieve no further significant increases. By the third week, the S_f 20-400 values are no longer significantly above control levels and have returned to normal at 21 days and at 47 days.

The S_f 0-20 class of lipoproteins becomes significantly elevated at 5 days and rises further over the next 2 weeks to a maximum level at 19 days. There is a decline after 19 days, but at 47 days the S_f 0-20 lipoproteins are still above control levels (Figs. 5 to 9).

Weight Change: Decrease in body weight follows a trend similar to that of the plasma lipid values (Fig. 10). No significant change in weight occurs during the first five days postexposure. Rapid fall in weight is observed from the seventh through the ninth

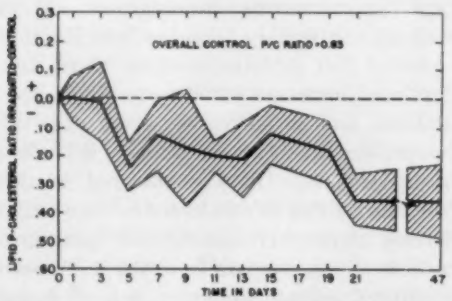

Fig. 7.—Alteration in lipid P-cholesterol ratio from controls after x-radiation ±2 standard deviations.

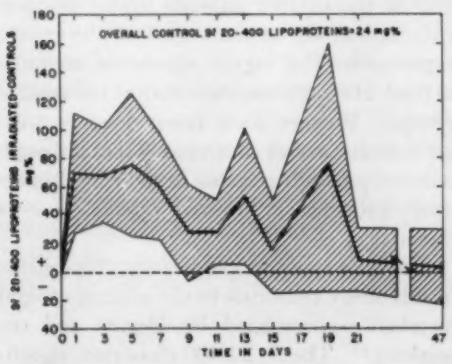

Fig. 8.—Departure in plasma S_f 20-400 lipoproteins from control levels after irradiation ±2 standard deviations.

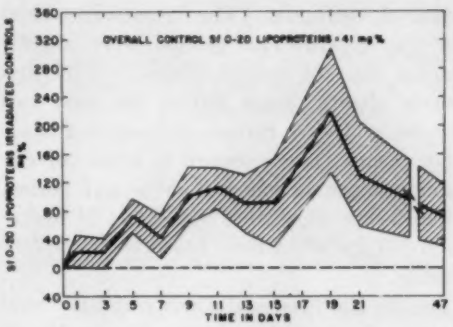

Fig. 9.—Departure in plasma S_f 0-20 lipoproteins from control levels after x-radiation ±2 standard deviations.

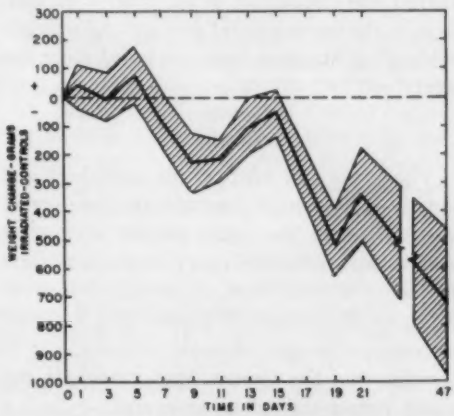

Fig. 10.—Body weight changes after x-radiation. Difference between experimental and control groups ±2 standard deviations.

day, the irradiated animals losing about 10% of their body weight in this period. A third phase occurs between the 9th and 15th days, characterized by an increase in body weight toward the preexposure mean level. A fourth phase supervenes around the 15th day, and the animals undergo marked weight losses to the 19th day, followed by further slight losses to the 47th day.

No quantitative balance studies were performed in this experiment. However, it appeared that the experimental animals consumed less rabbit chow and water than the control groups. This reduced intake was fairly uniform throughout the study, no distinct periods of anorexia or hyperorexia being observed. Hematocrits were performed in order to obtain some idea of each animal's

state of hydration. The hematocrit values in the experimental groups were slightly higher than the control values over the first week, slightly lower during the next two weeks, and were further reduced in the 47-day group. There seemed to be no correlation between hematocrit levels and plasma lipid levels, suggesting that states of hydration did not affect these blood values significantly.

Aside from reduced activity and the local injury changes, the irradiated animals evidenced no differences in appearance from the controls. The occasional diarrhea which occurred was commoner in the control animals than in the experimental groups. All animals evidencing diarrhea were excluded from the study.

COMMENT

The gross and microscopic pathology and muscle ATP and dry weight analyses correlate well with the usual picture seen after severe local radiation injury, namely, a latent period of several days, a period of destruction and dissolution of tissue, and a period of resorption and ultimate fibrosis. It is obvious that the plasma lipid values do not solely reflect this series of events.

During the first week postirradiation the increased lipid concentrations may be related to the breakdown of the limb tissues. The mechanism of this induced hyperlipoproteinemia is as yet obscure. The degradation, dissolution, and absorption of muscle protein might be responsible for the initiation of lipoprotein synthesis elsewhere in the body. The release of cholesterol and lipoproteins from the damaged tissues could itself contribute to the hyperlipoproteinemia. A third possibility is the actual increased synthesis of lipids by the injured muscle. In this regard, it has been reported from this laboratory that increased cholesterol levels are found in injured muscle tissue.[2]

The hyperlipidemia seems to recede between the 11th to the 15th day. A weight gain during this period deflects some indication of recovery of the animal generally, and the muscle sections also reveal beginning organization and repair. Thus, although the partial reversal of the hyperlipoproteinemia is not highly significant in this phase, independent data seem to corroborate it.

Significant rises in S_f 0-20 lipoprotein and cholesterol levels occur again during the third week after radiation, accompanied by marked weight loss. It is during this stage that breakdown of skin and extensive necrosis of subcutaneous tissues are occurring. The release of more products of tissue degradation into the general circulation may again be responsible for the second hyperlipoproteinemic response observed. Regions of necrosis are ideal sites for bacterial growth, and gross infection was observed locally in most of the injured animals. This complicating infection with its accompanying further tissue damage may also be a causative factor in the second elevation of plasma lipids.

The parallelism between weight loss and hyperlipoproteinemia is suggestive of an etiologic relationship. Aladjem and Rubin [11] observed that rabbits fasted for three days developed increased low-density serum lipoproteins, and on fasting for seven days developed further increases in the S_f 0-12 class of lipoproteins. It was observed in this laboratory † that rabbits fasted for two weeks develop elevated cholesterol and lipoprotein concentrations comparable to the levels seen in injured animals. However, animals fasted over this period lost about 30% of their original body weight, considerably more than that of the injured animals fed ad libitum. In addition, the animals used in the injury experiments did ingest significant amounts of food, although less than that of the control groups. Further work being done at present [12] demonstrates conclusively that although reduced intake is a factor in inducing hyperlipoproteinemia in injured rabbits, it is a minor one.

The time trend of low-density lipoproteins in this study conforms to the scheme of lipid metabolism postulated by Hewitt and coworkers.[13] The S_f 20-400 class rises signifi-

† Weiner, N., and Milch, L. J.: Unpublished data.

cantly on the 1st day and returns to normal levels by the 47th day. The S_f 0-20 class does not become elevated significantly until the fifth day and has not returned to normal levels at the termination of the experiment.

Depressed P/C ratios, a condition which presumably favors the development of atheromata,[14] were again observed after radiation injury and were most marked in the 21-day and 47-day groups. In this regard, one long-term group (three experimental animals surviving) was killed four months after radiation, at which time the cholesterol and S_f 0-20 lipoprotein elevations and the P/C ratio depression were still present. It is of interest to note that the one animal in this group which had overt infection at the stump of the irradiated limb had the highest cholesterol and lipoprotein values, again possibly indicating that active infection and necrosis is an important factor in the perpetuation of the hyperlipoproteinemia of injury. Postmortem examination of these animals failed to reveal atheromatous formations in the heart or aorta.

SUMMARY

Rabbits subjected to radiation injury of the right hindlimb are found to develop elevated plasma lipid levels during the first week postexposure, which persist for several weeks thereafter. The time curves of individual lipid and lipoprotein components are presented. Muscle ATP decreases progressively after the fifth day postirradiation to essentially zero levels by the 13th day. Progressive destruction of all tissues of the irradiated limb occurs over several weeks, eventually culminating in spontaneous amputation.

The statistical analyses were performed with the aid of Dr. W. F. Taylor and Mr. David Rubinstein, Department of Biometrics, School of Aviation Medicine, U. S. A. F., Randolph Field, Texas.

Irradiation was performed by A/1C John Wilkinson, Department of Radiobiology, School of Aviation Medicine, U. S. A. F., Randolph Field, Texas.

Histological sections were prepared and interpreted by Capt. Carl G. Hoak, Department of Pathology, School of Aviation Medicine, U. S. A. F., Randolph Field, Texas.

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Islet-Cell Tumor of the Pancreas with Hyperinsulinism

Failure of Surgical and Alloxan Treatment
Report of a Case

JUNE FISHER, M.D.
JOHN ARMES GIUS, M.D.
and
RALPH G. JANES, Ph.D., Iowa City

Functioning tumors of the islet cells of the pancreas produce excessive secretion of insulin resulting in hypoglycemia. There may be great variation in the symptoms produced by the hypoglycemia, but oftenest there are episodes of nervousness, trembling, sweating, weakness, unsteady gait, emotional instability, inability to concentrate, bizarre behavior, mental confusion, drowsiness, or unconsciousness with or without convulsions.

In 1950, Howard, Moss, and Rhoads[1] in a comprehensive review of islet-cell tumors collected 398 cases, 200 of which were operated upon for hyperinsulinism. In 81 of the cases no tumor was found at operation. Islet-cell tumors may occur at any age, but the peak incidence is between 40 and 50 years. There is no difference in sex incidence. They are encountered in about 1 out of 800 to 1000 autopsies.[2] Most of the tumors are small and functionless. They may occur in any portion of the pancreas and have also been found in the duodenal wall, the hilum of the spleen, the retropancreatic tissue, the gall bladder and stomach wall, and in Meckel's diverticulum.

The diagnostic criteria include:

1. Signs and symptoms consistent with insulin shock, which are usually progressive and which may be induced by fasting or exercise
2. Blood sugar levels below 50 mg. per 100 ml. during the attacks
3. Symptomatic relief following the administration of glucose

Surgical treatment is most effective, and exploration is indicated if the diagnosis of islet-cell tumor is suspected. Dietary management consists in providing a high-protein low-carbohydrate intake, divided into three main meals with interval feedings to maintain the blood sugar. Cortisone and corticotropin have also been used in hyperinsulinism for the temporary diabetic effect which they produce, but results have been variable.[3] Alloxan monohydrate has been administered to patients with both benign and malignant functioning tumors of the islet cells. Most recent investigators have reported only transient symptomatic relief or therapeutic failure.[4] One apparently satisfactory result was reported by Griffith, Jackson, and Janes.[5] Temporary improvement in hypoglycemic symptoms following administration of alloxan, 50 to 100 mg. per day for 20 days, was reported by Riva and Negri.[6]

REPORT OF CASE *

First Admission (Feb. 16, 1953): A 57-year-old janitor was admitted to the University Hospitals for recurrent episodes of confusion, jerking movements of the body, profuse perspiration, and disorientation, all of which occurred in the early morning hours (4 a. m. to 7 a. m.). The attacks had begun about three months before and had gradually

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From the Department of Internal Medicine, Surgery and Anatomy, State University of Iowa College of Medicine and the University Hospitals.

* Certain aspects of the present case have been reported in preliminary form.[7]

increased in severity. It had been observed that after breakfast the symptoms disappeared.

The past history was noncontributory except for mild asthma for 15 years and a cholecystectomy 5 years previously.

The physical examination was within normal limits, although it was noted that the patient appeared depressed and answered questions slowly. Hypoglycemia was suspected, and the patient was fasted for 24 hours. This produced a severe reaction manifested by bizarre shaking movements, excessive perspiration, and confusion. At this time the blood sugar was 50 mg/100 ml. (Table). Administration of dextrose intravenously relieved the reaction.

A glucose tolerance test gave the following values: 71, 153, 165, 180, 120 mg/100 ml. Other studies included lumbar puncture, encephalograms, skull films, Kepler water test, liver function studies, and blood chemistry determinations. All were within normal limits.

A functioning islet-cell tumor of the pancreas was suspected. The patient was discharged to the care of his local physician on a high-protein, low-carbohydrate diet with interval feedings.

Second Admission (Sept. 13, 1953): The patient was free of symptoms for nearly six months, but just prior to his second admission he had had several severe hypoglycemic attacks and could not continue working.

The fasting blood sugar level was 40 mg/100 ml.

Surgical treatment was advised, and the patient was explored on Sept. 14 through an upper abdominal transverse incision. Careful inspection and palpation of the pancreas, as well as other areas which could harbor pancreatic tissue, was carried out, but no tumor was found. About 40% of the pancreas, including the tail and body, was resected, but no tumor or other abnormalities were found in the excised specimen.

The postoperative course was uneventful. The fasting blood sugar level was 85 mg/100 ml. He was discharged in good condition on a diet similar to that which he had been given previously.

Third Admission (Oct. 14, 1953): The patient entered the hospital stating that his symptoms had returned. His fasting blood sugar level was now 17 mg/100 ml. The glucose tolerance test showed the following values: 36, 65, 157, 190, 153 mg/100 ml. (Fig. 1).

He was reexplored, but again no tumor was found. A subtotal pancreatectomy was carried out. It was estimated that about 90% of the pancreas was removed during the two operations.

Effect of Partial Pancreatectomy and Alloxan Therapy on Blood Sugar Levels

Date	Remarks	Blood Sugar Levels, Mg./100 Ml.	Alloxan Therapy, I. V.
2/16/53	First admission	50	.....
9/13/53	Second admission Before pancreatectomy	40	.....
9/16/53	Postpancreatectomy (40% of pancreas removed)	85	.....
10/14/53	Third admission	17	.....
10/27/53	Postpancreatectomy (90% of pancreas removed)	170	.....
12/ 9/53	Fourth admission Before alloxan	10	35 mg./kg. body wt. to 115 mg./kg.
1/18/54	After 1 mo. alloxan therapy	60	Total given during month, 1035 mg./kg.
2/12/54	Fifth admission	20	.....
3/ 2/54	.....	32	.....
3/ 7/54	After alloxan	78	Alloxan 125 mg./kg.
5/21/54	Sixth admission	29 15	.....
7/12/54	Seventh admission	39	Alloxan 125 mg./kg.
7/14/54	.....	..	Alloxan 150 mg./kg.
7/17/54	Patient died		

The postoperative course was again satisfactory, and the fasting blood sugar level was 170 mg/100 ml. His mental status appeared improved, and he was discharged on the 10th day.

Fourth Admission (Dec. 9, 1953): The patient was readmitted for recurrence of symptoms. The fasting blood sugar level was 10 mg/100 ml. The patient was somewhat lethargic, and he had evidence of thrombosis of the deep veins of the right leg. Intravenous alloxan therapy was started in amounts of 35 mg/kg. of body weight initially, with increase of the dosage gradually during a period of a month until he was receiving 115 mg/kg. (Table). Daily blood sugar determinations ranged from an initial low of 10 mg/100 ml. to a high of 60 mg/100 ml. at the end of treatment. He improved and was discharged with instruction to follow the previous diet.

Fifth Admission (Feb. 12, 1954): One month after the alloxan therapy the patient returned, complaining of early morning lethargy and confu-

sion. The fasting blood sugar determination at this time was 20 mg/100 ml., and the glucose tolerance test gave the following values: 15, 63, 117, 129, 89 mg/100 ml. (Fig. 1).

Because the initial blood sugar level was low, the dose of alloxan was increased to 125 mg/kg. of body weight. A single injection produced a severe reaction manifested by flushing of the skin and respiratory wheezing, which subsided within a few minutes. The fasting blood sugar levels during the following week ranged from 50 to 78 mg/100 ml. The patient seemed more alert and generally improved.

were now 39 and 40 mg/100 ml. A glucose tolerance test gave the following values: 23, 92, 123, 150, 123 mg/100 ml. (Fig. 1). Liver function studies were carried out and found to be within normal limits.

It was again decided to begin alloxan therapy because cortisone now appeared ineffective in maintaining the blood sugar levels. The drug was administered in the amount of 125 mg/kg. of body weight and caused no adverse reaction. The following day, 150 mg/kg. of body weight was given. At this time the patient complained of pain at the injection site and transient nausea.

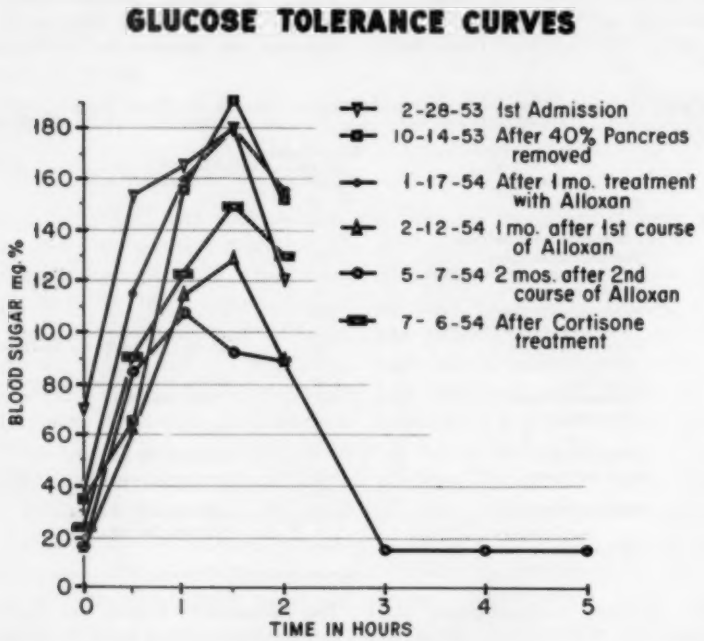


Figure 1

Sixth Admission (May 12, 1954): The patient was readmitted two and a half months later for recurrent symptoms of hypoglycemia. At this time the fasting blood sugar level was 15 mg/100 ml. He was given two intravenous injections of glucagon, 20 and 40 units per kilogram of body weight, respectively, without observable effects on the blood sugar. Cortisone, 25 mg. orally four times daily, was prescribed, and this therapy resulted in some elevation of the blood sugar. A glucose tolerance test showed the following values: 15, 85, 109, 92, 89, 15, 15, 15 mg/100 ml. (Fig. 1).

Seventh Admission (July 12, 1954): During the preceding month the patient received cortisone, 25 mg. three times daily, and had been able to do some physical labor. He had had no episodes of mental confusion. The fasting blood sugar levels

A needle biopsy of the liver was performed, and the sections appeared to be essentially normal. The liver cells contained considerable amounts of glycogen, and there was some fatty metamorphosis but there was no evidence of parenchymatous degeneration (Fig. 5).

About 24 hours later the patient suddenly went into shock. The hypotension was reversed by means of whole blood transfusions and an intravenous drip of arterenol. However, he soon developed jaundice which was followed by fever (temperature 106 F), dyspnea, cyanosis, and irreversible shock with coma, and he died four days after the last injection of alloxan.

At autopsy, an islet-cell tumor was found embedded in the small remaining segment

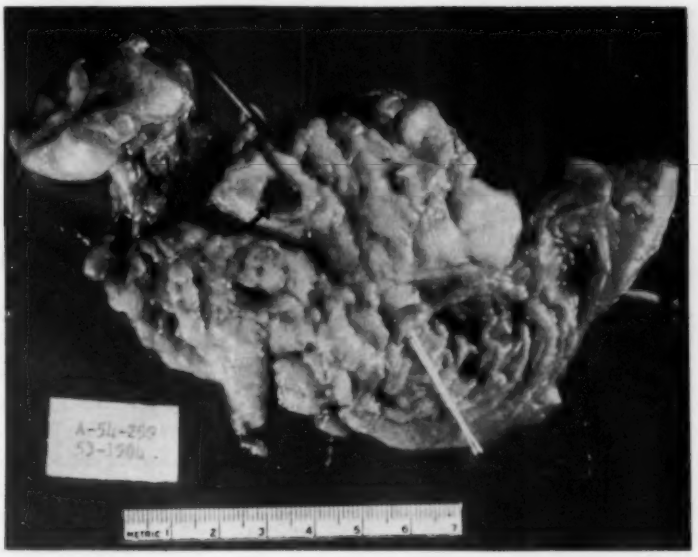
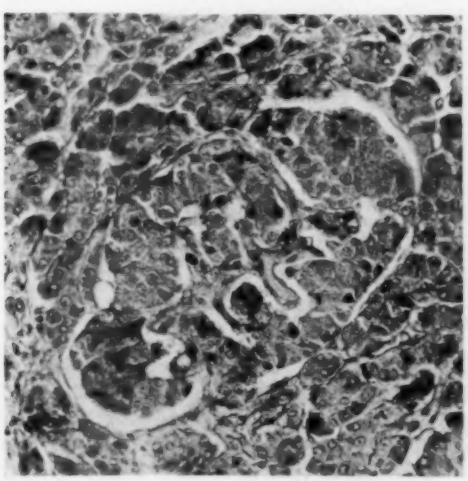


Fig. 2.—Photograph of gross specimen. The probe is in the common bile duct. The islet-cell tumor is indicated by the arrow.

of pancreatic tissue, about 1.5 cm. from the sphincter of Oddi. It measured 1.2×1.0×0.8 cm. (Fig. 2). The tumor, together with normal pancreas, was fixed in Bouin's fluid, embedded in paraffin, and cut at 4μ. The sections were oxidized with permanganate and stained with the aldehyde-fuchsin technique. The tumor was well encapsulated by fibrous connective tissue, and bundles of collagenous fibers extended into the tumor, dividing it into irregular clusters of cells (Fig. 3). β-Granules, although not abundant, were found in some of the cells. This is in agreement with Nerenberg.[8] In general, the cells in the center of the clusters had a lighter staining cytoplasm. The nuclei of the tumor cells were round or slightly oval, contained fine chromatin granules, and usually a small nucleolus. However, the nuclei of many of the cells, particularly those on the periphery of the clusters, showed a hyperchromatosis or were pyknotic. Except for this, there was no evidence of degeneration of the tumor cells.

Fig. 3.—Section of islet-cell tumor stained by the aldehyde-fuchsin technique. The tumor cells were arranged in irregular clusters. Many nuclei, particularly at the periphery of the clusters, were pyknotic or showed hyperchromatism.



The sections of the remaining pancreas were not remarkable. The acinar tissue appeared normal and, although the islets were not numerous, those present were quite large (Fig. 4). β-Granules were found in some of the islet cells. Actual necrosis of the β-cells, which is observed following the administration of alloxan to experimental animals, was not seen. On the other hand, rather bizarre mitotic figures were found in many of the islet cells, as many as eight in one islet.

Acute parenchymatous changes were found in the kidneys, liver, and adrenals. The distal segments of the nephron units showed considerable tubular epithelial damage. Edema

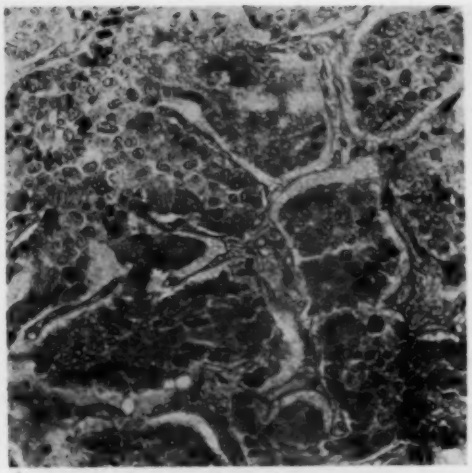


Fig. 4.—Section of normal islet from intact pancreas stained by the aldehyde-fuchsin technique. β-granules were present in some of the cells. In addition, rather bizarre mitotic figures have been found in many of the islets, but they cannot be seen under this magnification.

was prominent. The interstitial tissues were infiltrated with neutrophiles, lymphocytes, and histiocytes. Hyaline and pigmented casts were found in the nephron and collecting tubules. The liver showed extensive infiltration with fat and degeneration and necrosis of central areas. Allochrome staining procedures showed little glycogen content. The changes in the kidney, liver, and adrenals could have been caused by shock and the postmortem state.

COMMENT

The patient received a prolonged trial on a high-caloric, high-protein, low-carbohydrate diet, with interval feedings, with little improvement. He continued to have lethargy and mental confusion, especially in the early morning hours, and the fasting blood sugar values ranged from 10 to 40 mg/100 ml. During the dietary treatment he gained about 30 lb. (13.6 kg.). Activity appeared to increase lethargy and mental confusion. Because the diet did not control his symptoms and because there was progression of the disease, he was subjected to exploration for functioning islet-cell tumor on two occasions. On each occasion, no tumor was found and partial pancreatectomy was carried out. After each partial pancreatectomy the patient experienced a remission of symptoms for several weeks with improvement in blood sugars from 10 mg/100 ml. prior to operation to 85 mg/100 ml. and 170 mg/ml. after the operations. Careful examination of the estimated 90% of pancreatic tissue removed failed to reveal any evidence of islet-cell tumor or hyperplasia.

Alloxan therapy was started two months after the last operation, when the symptoms of hypoglycemia had become uncontrollable. The side-reactions noted from the intravenous administration of alloxan, 125 mg/

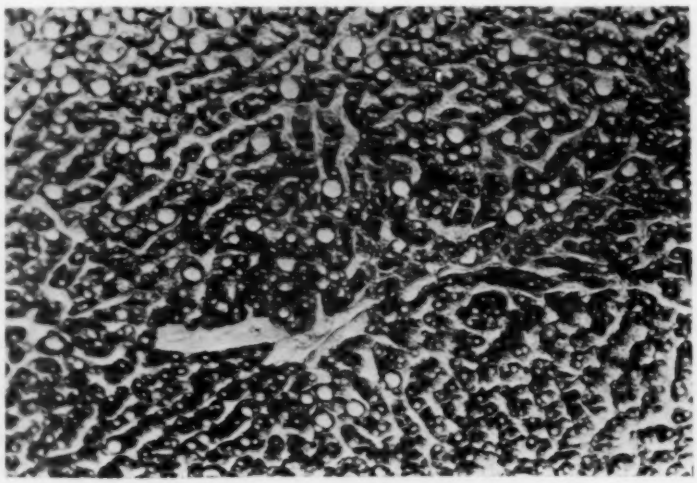


Fig. 5.—Section of liver from biopsy taken five days before death. Hotchkiss-McManus preparation. The liver structure was quite normal, although there was some fatty metamorphosis. The liver cells contained considerable amounts of glycogen.

kg. of body weight, included pain, redness, swelling at the site of injection with immediate sclerosis of veins, and generalized transient flushing of skin with respiratory distress and wheezing. Temporary improvement was noted after alloxan therapy but could not be maintained. There was no evidence of liver or kidney impairment from alloxan until the terminal illness, which was acute and fulminating. At the time of the liver biopsy, alloxan in the amount of 125 mg/kg. of body weight already had been given without adverse clinical effects. The biopsy had revealed essentially normal liver tissue.

The question thus arises as to what effect alloxan had on the pancreas. Three points seem worthy of mention. First, it is obvious that alloxan, in the doses administered, failed to destroy β-cells both in the tumor and in the normal islets. Second, the presence of pyknotic nuclei in some of the tumor cells might indicate that the alloxan caused some damage. On the other hand, this might indicate early degenerative changes in the tumor *per se*, which had nothing to do with the alloxan. Third, the presence of abnormal mitotic figures in the β-cells in the normal islets might be a response to the alloxan therapy or an attempt, on the part of the islets, to produce more tumor masses.

The exact cause of death in this patient is somewhat obscure. At necropsy there was an acute parenchymatous nephritis and evidence of a mild pyelonephritis, as well as some parenchymatous degeneration of the liver and adrenals. After the last dose of alloxan (150 mg/kg. of body weight), however, the patient had an acute hemolytic episode, became jaundiced, and went into shock. Four days later he died. The pathological changes seen in the kidney, liver, and adrenals are compatible with those which may occur from shock. It is unlikely that the liver was damaged by the alloxan *per se*. Other authors † have reported the administration of much higher doses of alloxan without evidence of toxic changes in liver, kidney, or adrenals in humans, although such changes have been reported in experimental animals.

† References 9 and 10.

SUMMARY AND CONCLUSIONS

A case of hyperinsulinism due to a benign functioning islet-cell tumor is reported. Both medical and surgical treatment did not halt the progress of the disease. The tumor was not found on two successive abdominal explorations in which about 90% of the pancreas was removed. Other therapeutic measures, including a high-protein, low-carbohydrate diet and cortisone, gave only temporary relief. Alloxan also produced only a temporary elevation of blood sugar and symptomatic relief. The local side-reactions of alloxan treatment included the following: pain, swelling, and redness, together with sclerosis of the veins, and, in some instances, a thrombosis at the site of injection. The generalized reactions, when large doses were given, consisted of the following: flushing, nausea, and wheezing respirations.

Death occurred four days after an alloxan injection of 150 mg/kg. of body weight. The injection was followed by shock, dyspnea, cyanosis, high temperature, jaundice, coma, and death.

At autopsy a small islet-cell tumor was found deep within the remaining pancreatic tissue in close proximity to the common bile duct. There was no histologic evidence of necrosis of the islet cells either of the normal pancreas or tumor which could be attributed to alloxan.

Other visceral changes, including parenchymatous degeneration of the liver, adrenals, and kidneys, were considered to be due to shock and the postmortem state.

Alloxan in the doses used appeared to have only a temporary inhibiting effect on insulin production by the functioning islet-cell tissue. There is reason to believe that alloxan in large doses may have toxic effects on other organs. At the present time the only sure method of controlling hyperinsulinism caused by a functioning islet-cell tumor is by the surgical removal of the tumor.

In this instance, total pancreatectomy would have been required.

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Congenital Goiter

Report of a Fatal Case with Postmortem Findings

WILLIAM G. BERNHARD, M.D.
HAROLD GRUBIN, M.D.
and
GEORGE A. SCHELLER, M.D., Newark, N. J.

Sporadic cases of congenital goiter have been reported with fair regularity in the North American literature. However, only 12 of the patients who were brought to necropsy * (Table 1) and 6 who were treated surgically † (Table 2) had adequate histopathologic study of the thyroid gland. Our case is of particular interest because (1) the weight of the gland (75 gm.) in our patient is greater than that of any in the previous cases, (2) the mother took an iodine preparation daily during her prenatal period, and (3) polyhydramnios was present. Death of the infant was caused by asphyxia resulting from pressure on the trachea by the enlarged thyroid gland.

REPORT OF CASE

A 41-year-old white tertigravida, primipara, was admitted to the Hospital of St. Barnabas and for Women and Children, on Nov. 14, 1952, for an elective induction of labor because of polyhydramnios. The baby died 1 hour 15 minutes after birth. Labor was uneventful, and the delivery was easy and spontaneous.

In 1940, the mother had had a basal metabolic rate of +26, with a pulse rate of 90. She was told she had mild hyperthyroidism which needed no treatment. Her first pregnancy was in 1949. It terminated in an uneventful and a spontaneous normal delivery. Her second pregnancy was in 1950 and terminated in a spontaneous abortion after two months' gestation with no apparent cause. As soon as the present pregnancy was diagnosed in May, 1952, the patient was started empirically on 5 drops of a saturated solution of potassium iodide three times daily. Thus, a total of 3 oz. (90 gm.) of the preparation was ingested during the last seven months of gestation. The basal metabolic rate on Dec. 17, 1952, was +22.

POSTMORTEM FINDINGS

The body was that of a baby girl with extreme edema of the face and a large mass in the neck (Fig. 1). The total body length was approximately 51 cm., and the body weight approximately 3450 gm.

The mass in the neck was an extremely large thyroid gland firmly adherent to the trachea. The lateral lobes completely encircled the trachea, with the tips adherent to the tracheal grooves. The inferior and superior thyroid veins and the external jugular veins were tremendously engorged. The gland was symmetrically enlarged, each lobe measuring approximately 5×5×2 cm., and the isthmus approximately 1.5×2 cm. The entire gland weighed 75 gm. The cut surface showed incomplete fibrous bands which divided the parenchyma into indistinct and incomplete lobules. Gross examination of the other organs was not contributory.

Microscopic sections of the thyroid gland were composed of extremely hyperplastic follicles completely devoid of colloid (Figs. 2 and 3). These acini were lined by faintly acidophilic and slightly granular columnar cells with exceedingly indistinct cytoplasmic borders. The acinar lining was disrupted and poorly preserved, giving a desquamated and pseudopapillary appearance. The nuclei were small to medium in size and round to polyhedral in shape. Some of them were deeply hyperchromatic, and others had the chromatin dispersed in fine granules. The follicles were incompletely divided by heavily congested bands of connective tissue of varying thickness.

Sections of other organs showed a diffuse hyperemia with no other significant changes.

Submitted for publication July 20, 1955.

From the Departments of Pathology and of Obstetrics, Hospital of St. Barnabas and for Women and Children.

* References 1 to 11.

† References 7 and 12 to 16.

TABLE 1.—*North American Congenital Goiter with Necropsies*

Reference	Maternal Goiter	Prenatal Iodine	Presence of Hydramnios	Survival Period	Size of Thyroid Gland	Microscopic Appearance
Abt, 1900	Not stated	None	Not stated	Died soon after birth	Size of walnut	Vascular and hyperplastic
Skinner, 1928	None	None	Not stated	7½ hr.	16.3 gm.	Not given
Mitchell and Struthers, 1933	Not stated	No maternal history	Not stated	6 wk.	12 cm. in circumference	Fibrosis and hyperplasia
Hill, 1933	Present	None	Not stated	4 days	57 gm.	Hyperplasia with colloid
Warren and Shpiner, 1937	Thyrotoxicosis	Yes	Not stated	Stillborn	Each lateral lobe 1.3×0.7×0.4 cm.	Hyperplasia with colloid
Solis-Cohen and Steinbach 1939	Myxedematous appearance	None	Not stated	48 hr.	41 gm.	Hyperplasia
Davies, 1943	Present	None	Not stated	13 days	Each lobe 4 cm. in diameter	Marked hyperplasia
Jones, 1951	None	None	Present	½ hr.	50 gm.	Marked hyperplasia
Jones, 1951	None	None	Present	5 hr.	20 gm.	Marked hyperplasia
Seligman and Pescovitz, 1950	Thyrotoxicosis	Yes	Present	½ hr.	16 gm.	Marked hyperplasia
Moran, 1952	Myxedema	None	None	14 days	12 gm.	Marked hyperplasia
Lincoln, 1953	None	None	Not stated	40 hr.	30 gm.	Hyperplasia with colloid
Author's case	Mild thyrotoxicosis	Yes	Present	1¼ hr.	75 gm.	Marked hyperplasia

COMMENT

Skinner [2] concludes that any mother taking a small dose of iodine at stated intervals during the gestation period can be absolutely assured that her baby will not be born with a goiter. Crile [17] recommends the prophylactic administration of 1 minim (0.06 cc.) once a week of a 25% solution of potassium iodide during pregnancy for the prevention of congenital goiter. Congenital goiter in endemic goiter regions is practically nonexistent since the advent of prophylactic prenatal iodine therapy. However, in addition to the patients brought to necropsy and those operated on, several clinical cases of congenital goiter have been reported in which the mother received iodine prenatally.‡ If we accept lack of iodine as the etiologic factor, then our case, along with other sporadic cases from nonendemic areas, suggests the basic mechanism in the production of such a goiter to be either poor assimilation of iodine or some defect in the cellular metabolism of iodine.

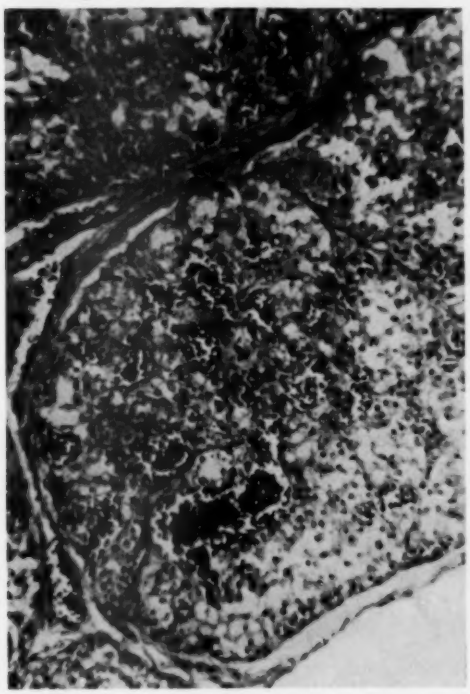
‡ References 18 to 20.

TABLE 2.—*North American Congenital Goiter Treated Surgically*

Reference	Maternal Goiter	Prenatal Iodine	Presence of Hydramnios	Age at Time of Operation	Thyroid Gland Removed	Microscopic Appearance
Peterson and Sondern, 1911	No	Not stated	Not stated	5 wk.	6×4×3 cm. and 4×2.5×2 cm.	Adenomatous proliferation with colloid
Williamson, 1913	Palpable thyroid	None	Not stated	18 mo.	25.5 gm.	Diffuse hyperplasia
Symmers, 1941	Not stated	Not stated	Not stated	6 wk.	47 gm.	Hürthle cell tumor
Davies, 1943	Small to medium goiter	Yes	Not stated	12 days	6 gm.	Subinvolution of hyperplastic gland
Morrow, 1945	Not stated	Not stated	Not stated	2 mo.	4×2×1.5 cm.	Hürthle cell tumor
Schifrin and Hurwitt, 1951	Not stated	None	Not stated	7 wk.	65 gm.	Diffuse hyperplasia

Fig. 1.—Swelling of neck and massive edema of face.

Fig. 2.—Pseudopapillary hyperplastic thyroid gland. Hematoxylin and eosin; reduced about ⅔ from mag. × 150.



We wish to call attention to the presence of polyhydramnios in our case. This condition is frequently associated with congenital defects of the newborn infant and has been reported in three other cases of fatal congenital goiter.

The histopathology of congenital goiter was adequately described by Korpássy.[21] Although textbooks differentiate three forms, namely, parenchymatous, telangiectatic, or colloid, he did not find any struma containing colloid in his series. Furthermore, he found capillary hyperemia, bleeding, marked desquamation, and hyperplasia of the epithelium so that so-called struma congenital telangiectasia cannot be considered as an independent form. The absolute absence of colloid is significant for the goiter of the newborn infant independent of the degree of enlargement, which is always diffuse. The swelling of the thyroid in the newborn infant is rarely caused by mechanical factors or by a generalized hyperemia, but by ex-

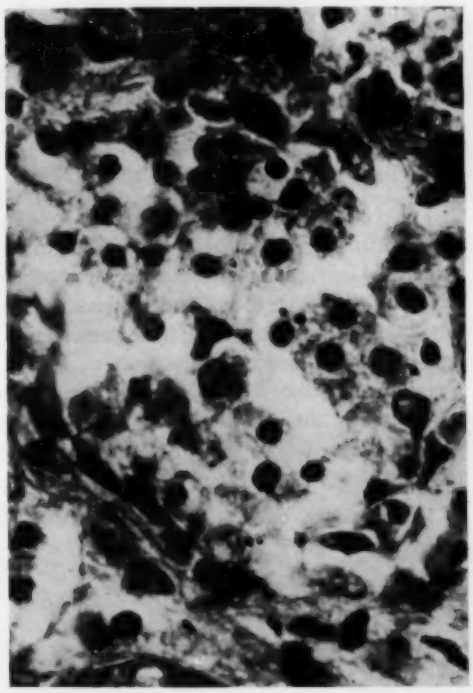


Fig. 3.—Desquamation of acinar epithelial cells, interstitial hyperemia, and absence of colloid. Hematoxylin and eosin; reduced about ⅔ from mag. × 300.

treme hyperplasia of the epithelium of the follicle.

All the cases reported in the North American literature with microscopic study, including our own, uniformly showed an epithelial hyperplasia. In nongoitrous areas of the United States the thyroid of the newborn infant rarely weighs less than 1.5 gm. The normal weight of the thyroid gland of the newborn infant seldom exceeds 3 gm., and microscopic study usually shows the presence of colloid in the acini.[22] The weight of the gland (75 gm.) in our patient is greater than that of any in the previously reported surgical and postmortem cases.

SUMMARY

The 13th fatal case of congenital goiter with postmortem study reported in the North American literature is presented. The mother was slightly thyrotoxic and received prenatal iodine therapy. Polyhydramnios was present. Microscopically the goiter showed a uniformly diffuse epithelial hyperplasia. The death of the infant was due to asphyxia or suffocation from pressure of the enlarged thyroid gland.

Dr. Edwin O. Gilbert and Mr. Bernhardt V. Lind, of the Bureau of Pathology, New Jersey State Department of Health, prepared the photographs.

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Regeneration of Pancreatic Acini During Ethionine Administration

THOMAS D. KINNEY, M.D.
NATHAN KAUFMAN, M.D.
and
JANIS V. KLAVINS, M.D., Cleveland

Pancreatic damage following the administration of DL-ethionine (α-amino-γ-ethylmercaptobutyric acid), a methionine antagonist, has been described by many workers.* The impression obtained is that degeneration of acini is progressive so long as the animals are fed diets containing ethionine. However, in the course of various studies on the effects of ethionine it was noted that there were limitations to the pancreatic damage; during relatively prolonged exposure to ethionine many of the pancreatic acini regenerated to such a degree that they resembled the functioning acini seen in the control groups.

MATERIALS AND METHODS

Fifty-five albino rats of the Sprague-Dawley strain, each weighing approximately 220 gm., were used. They were fed ad libitum. Nineteen rats served as controls and received a synthetic diet of the following composition: glucose 67 gm.; vitamin-free casein 18 gm.; salt mixture 4 gm.[7]; corn oil 11 gm., containing 0.001 cc. halibut liver (Haliver) oil. Crystalline vitamins were added to supply the following amounts per 100 gm. of diet: thiamine chloride 400γ, pyridoxine hydrochloride 400γ, riboflavin 800γ, calcium pantothenate 1.5 mg., and nicotinic acid 2.5 mg. The remainder of the animals were fed an identical diet, to which was added 0.5% DL-ethionine. Twenty-eight animals were kept on the ethionine-supplemented diet continuously for 10, 28, and 63 days, at which times they were killed and autopsied, along with corresponding controls. In addition, eight animals received an ethionine-supplemented diet for 28 days and then were returned to the control diet for the remainder of the 63-day period. This was done in order to compare their pancreases with those of the animals on a continued ethionine diet during the same experimental period. Portions of the pancreases were fixed in formalin, sectioned, and stained with hematoxylin and eosin stain and Van Gieson's stain for connective tissue.

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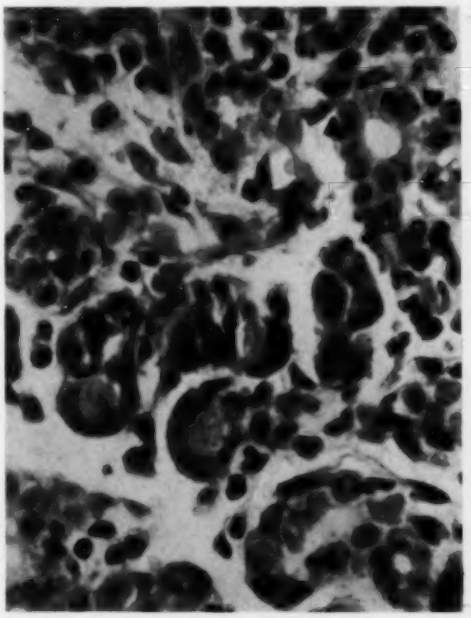
From the Departments of Pathology, Cleveland City Hospital and Western Reserve University School of Medicine, Cleveland, and Marymount Hospital, Garfield Heights, Ohio.

* References 1 through 6 and 9 through 17.

RESULTS

After the animals were fed ethionine for 10 days, there was striking pancreatic dam-

Fig. 1.—Pancreas, 10 days' ethionine feeding. Diffuse acute pancreatitis. Loss of normal acinar architecture, infiltration by polymorphonuclear leucocytes and lymphocytes, and slight fibroblastic proliferation. Hematoxylin and eosin; reduced slightly from mag. ×750.



age (Fig. 1). The changes were similar to those previously described.† These consisted of loss of normal acinar structure, cellular disintegration, vacuolation of the cytoplasm of the acinar cells, disappearance of zymogen, loss of basophilia, and, occasionally, chromatolysis, karyorrhexis, and pyknosis of the acinar cells. There was infiltration by polymorphonuclear leucocytes and lymphocytes and slight fibroblastic proliferation. Occasionally interstitial edema was present. After 28 days of ethionine administration the pancreatic damage in some animals was marked (Fig. 2) and was similar to that seen after 10 days, although the inflammatory component was less striking. However, in other animals of the 28-day group the pancreases showed evidence of active regeneration in addition to areas of atrophy. These regenerating acinar cells were close together and contained very little zymogen and abundant basophilic vacuolated cytoplasm. The nuclei were large and hyperchromatic, with prominent single or multiple nucleoli. Occasionally, mitotic figures were seen (Fig. 3). In some areas the nuclei as well as the cells were closely crowded.

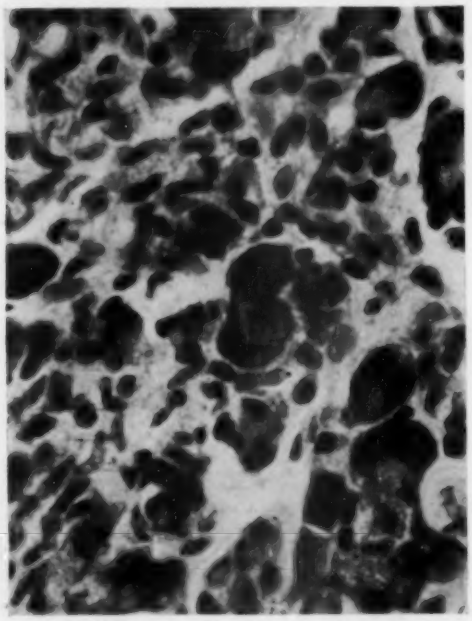


Fig. 2.—Pancreas, 28 days' ethionine feeding. Pancreatic damage and cellular infiltration similar to that at 10 days except for the absence of polymorphonuclear leucocytes. Hematoxylin and eosin; reduced slightly from mag. × 750.

After 63 days there was not only regeneration but also further differentiation of the regenerated acini. Here, the differentiated acini closely resembled those of the control animals (Fig. 4), although they were reduced in number. In these acini there was abundant zymogen as well as basal perinuclear basophilia similar to that seen in the controls. In other small areas there was still some evidence of active regeneration, and the cells resembled those in the animals killed after 28 days. Some few mitotic figures were still present in such areas (Fig. 5). In association with the decrease in acini there was an increase in the interstitial fat and connective tissue (Fig. 5*B*).

In the islets of Langerhans occasional slight cytoplasmic vacuolation and hyperemia were observed in all animals receiving ethionine.

In the group of rats that received ethionine for 28 days and then were fed the control diet lacking in ethionine for the rest of the experimental period, the acini were almost completely restored. Prominent nucleoli and a few mitotic figures were still present, and the regenerated acini resembled very closely the regenerated differentiated acini seen in the group that had been on the ethionine diet for the entire 63-day period, but there was no appreciable increase in the connective tissue.

The initial loss of weight in the experimental animals was striking. At 10 days the weight change was such that the animals had lost 18.7% of their initial weight, in contrast with a gain of 13.5% in the control group. After this period there was an increase in weight, so that in the next 18 days (that is, by the 28th day of the experiment) the average weight loss was 4.1% and by 63 days it was only 1.1%, when compared with their weight at the beginning of the experiment.

† References 1 through 6 and 9 through 17.

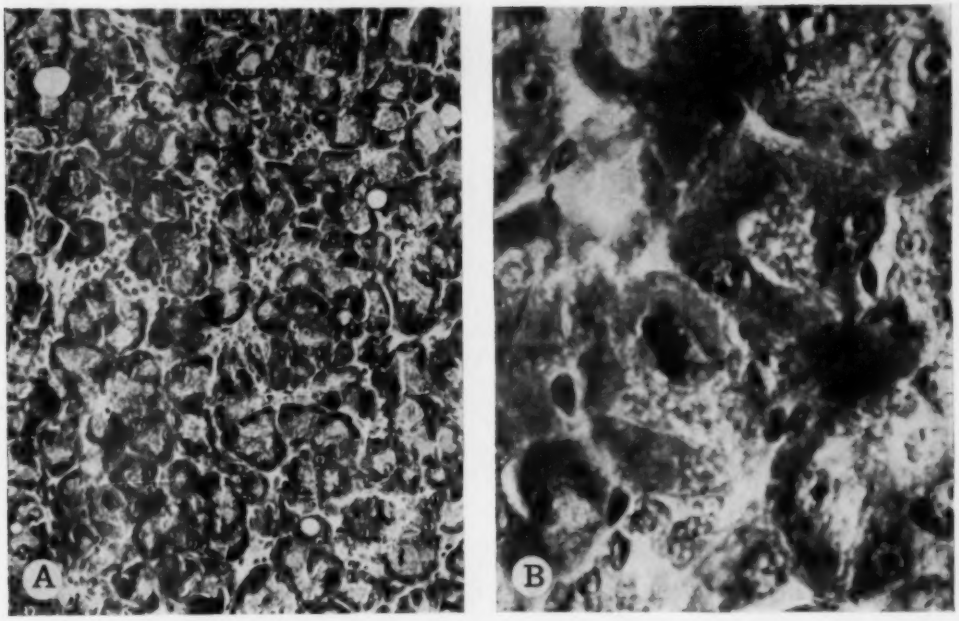
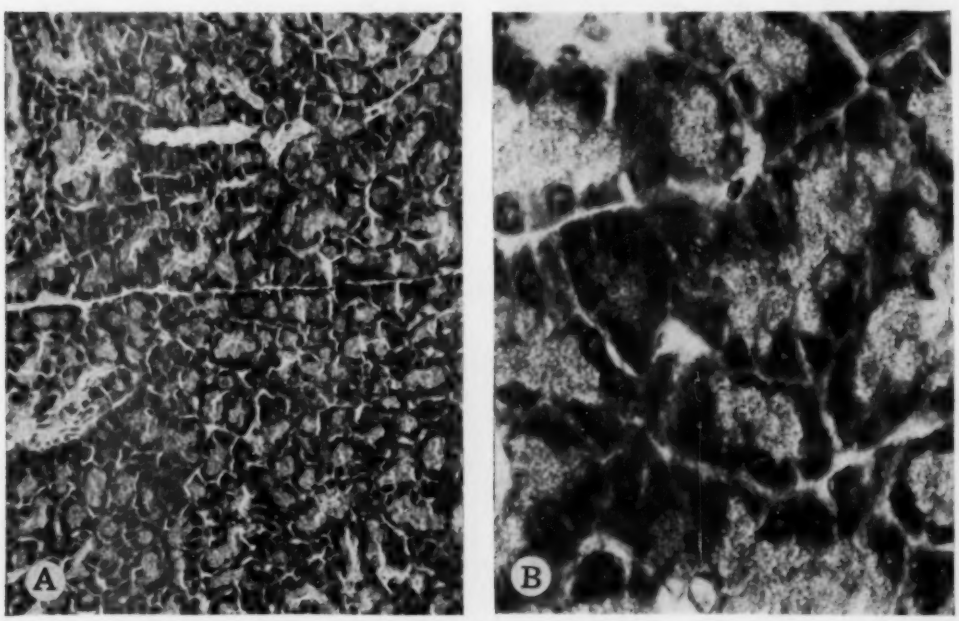
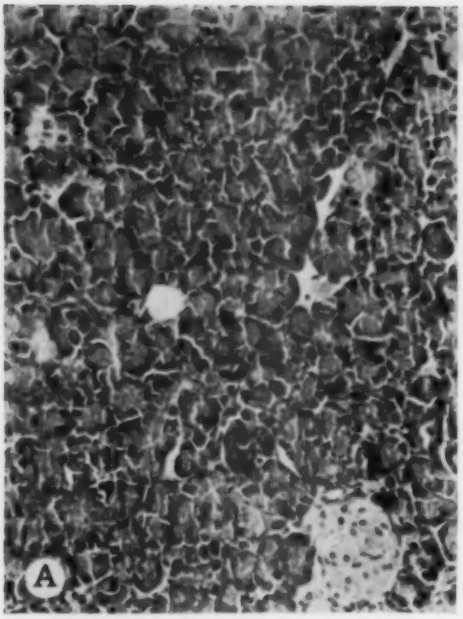
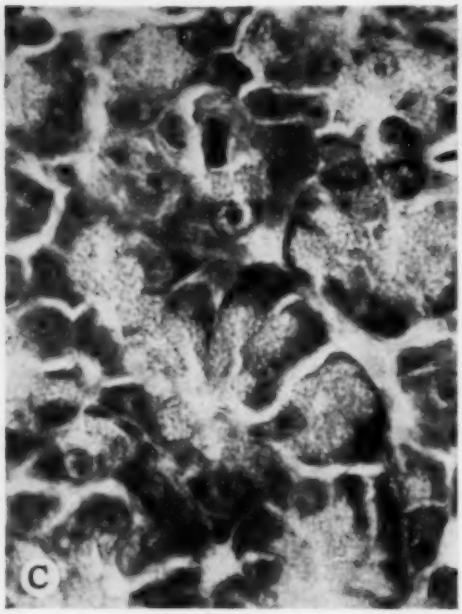


Fig. 3.—Regenerating pancreas, 28 days' ethionine feeding. Hematoxylin and eosin. *A*, prominent single and multiple nucleoli. Mitotic figures are present. Reduced slightly from mag. ×175. *B*, mitotic figure in the center. Notice prominent single and multiple nucleoli. Reduced slightly from mag. × 812.

Fig. 4.—Normal rat pancreas. Control (for comparison with Fig. 5). Hematoxylin and eosin. *A*, reduced slightly from mag. × 175. *B*, × 750.







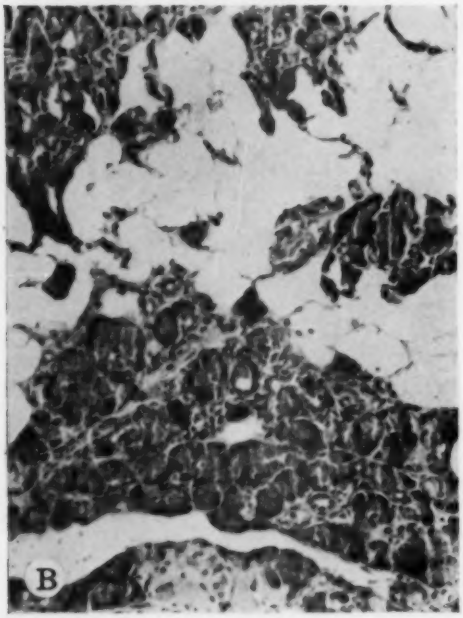


Fig. 5.—Pancreas, 63 days' ethionine feeding. Hematoxylin and eosin. *A*, in this field the acini are closely grouped and the architecture resembles the normal. Compare with Figure 4*A*. Reduced slightly from mag. × 175. *B*, section from another animal in the same group shows a decrease in the number of acini which appear normal. There is an increased amount of fat and interstitial connective tissue. Reduced slightly from mag. × 175. *C*, regenerated acini showing close resemblance to normal. A mitotic figure is present. Reduced slightly from mag. × 750.

COMMENT

Methionine appears to be essential for the healthy maintenance of the pancreas, and lack of it leads to damage of the acini. This is demonstrated by the administration of its antagonist, ethionine. However, during and following exposure to ethionine some of the pancreatic acini regenerate. After four weeks the regeneration is quite advanced, although many of the acini are poorly differentiated. After two months regeneration is almost complete, and the regenerated acini closely resemble the normal.

Fitzgerald and Alvizouri [4] reported regeneration of the pancreas after ethionine withdrawal. Wachstein and Meisel [17] also reported regeneration of the pancreas after withdrawal of ethionine. These changes were similar to those seen by us, even though our animals were still on the ethionine diet. Popper and co-workers [14] mention pancreatic damage followed by abnormal regeneration of the pancreas following four weeks of ethionine administration.

When the weight changes are compared with the pancreatic damage, it is of interest

that the greatest weight loss coincides with the early acute stage of pancreatic damage. As regeneration progresses, there is a gradual gain which is quite similar to that in the controls. Henning and Heinkel ‡ in their study of the plasma diastase in ethionine-treated rats found that there was immediate decrease of plasma diastase level after injection of DL-ethionine. An abnormal increase was noticed a day later, and the values were normal on the sixth day after a single administration of ethionine. These observations raise the possibility of a return of function concomitant with acinar regeneration.

SUMMARY

After administration of ethionine there is acute pancreatic damage followed by regeneration. Restitution of single pancreatic acini to normal appearance is present after prolonged ethionine feeding.

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Histopathology of the Irradiated Hibernating Ground Squirrel

FRANK W. FITCH, M.D., Wichita Falls, Texas
JOHN DOULL, Ph.D.
and
ROBERT W. WISSLER, M.D., Chicago

INTRODUCTION

Hibernation prolongs survival in ground squirrels (Citellus tridecemlineatus) when they are given whole-body x-irradiation while in hibernation and then are maintained in the hibernating state after irradiation. The ultimate mortality remains unaltered, however.[1] Survival of frogs is greatly enhanced if the animals are kept in the cold continuously after exposure to x-rays.[2] This increased survival appears to be related to a slowing of the development of cellular damage.* A study of the ground squirrel after irradiation was undertaken to determine whether similar retardation of cellular damage occurred as a result of hibernation. The experiments reported in this paper represent a histological study of the normal hibernating and nonhibernating ground squirrel and of the pathological changes developing after whole-body x-irradiation in each physiological state.

MATERIAL AND METHODS

Male and female ground squirrels were trapped in the vicinity of Chicago during the summer months by placing a wire cage over the entrance to the burrow and flooding the burrow with water, thus forcing the animals into the cage. They were maintained in an air-conditioned laboratory at 68 to 75 F in individual cages and were fed Rockland Rat Diet supplemented biweekly with carrots. Hibernation was induced in adult animals by placing them in a cold room at 5 C for 24 to 48 hours without food or water. Hibernation in this species is more profound than in other available hibernating animals. It is not, however, always continuous. Animals apparently in deep hibernation may awaken for a short time and then resume hibernation.[5]

X-irradiation was administered by means of a Keleket therapy x-ray machine operating at 250 kvp and 15 ma. with 0.25 mm. Cu and 1 mm. Al added filtration. Target distance was 75 cm. and dose rate averaged 35 r/minute as calibrated with a Victoreen ionization chamber in air. Groups of 12 animals were irradiated simultaneously in a rotating aluminum cage. When animals were irradiated while in the hibernating state, the cage was maintained at 5 C during the exposure. Animals were given a single exposure of 800 r to the entire body, which has been found to cause 80% mortality in normal adult nonhibernating ground squirrels within 10 days, and 100% mortality within 30 days, after irradiation.[1]

Animals were killed by exsanguination after being anesthetized with ether. Tissues were fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with the azure A-eosin B method.

RESULTS

Three nonhibernating ground squirrels were killed for examination of normal histology. Groups of three ground squirrels each were killed after one week and five weeks in deep hibernation, and three days after awakening from five weeks of deep hibernation. Groups of three nonhibernating ground squirrels each were killed six hours, one day, and three days after 800 r whole-body x-irradiation. Groups of three animals each were killed 6 hours, 1 day, 3 days, and

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From the Departments of Pathology and Pharmacology of the University of Chicago.

Public Health Service Research Fellow of the National Microbiological Institute (Dr. Fitch).

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* References 2, 3, and 4.

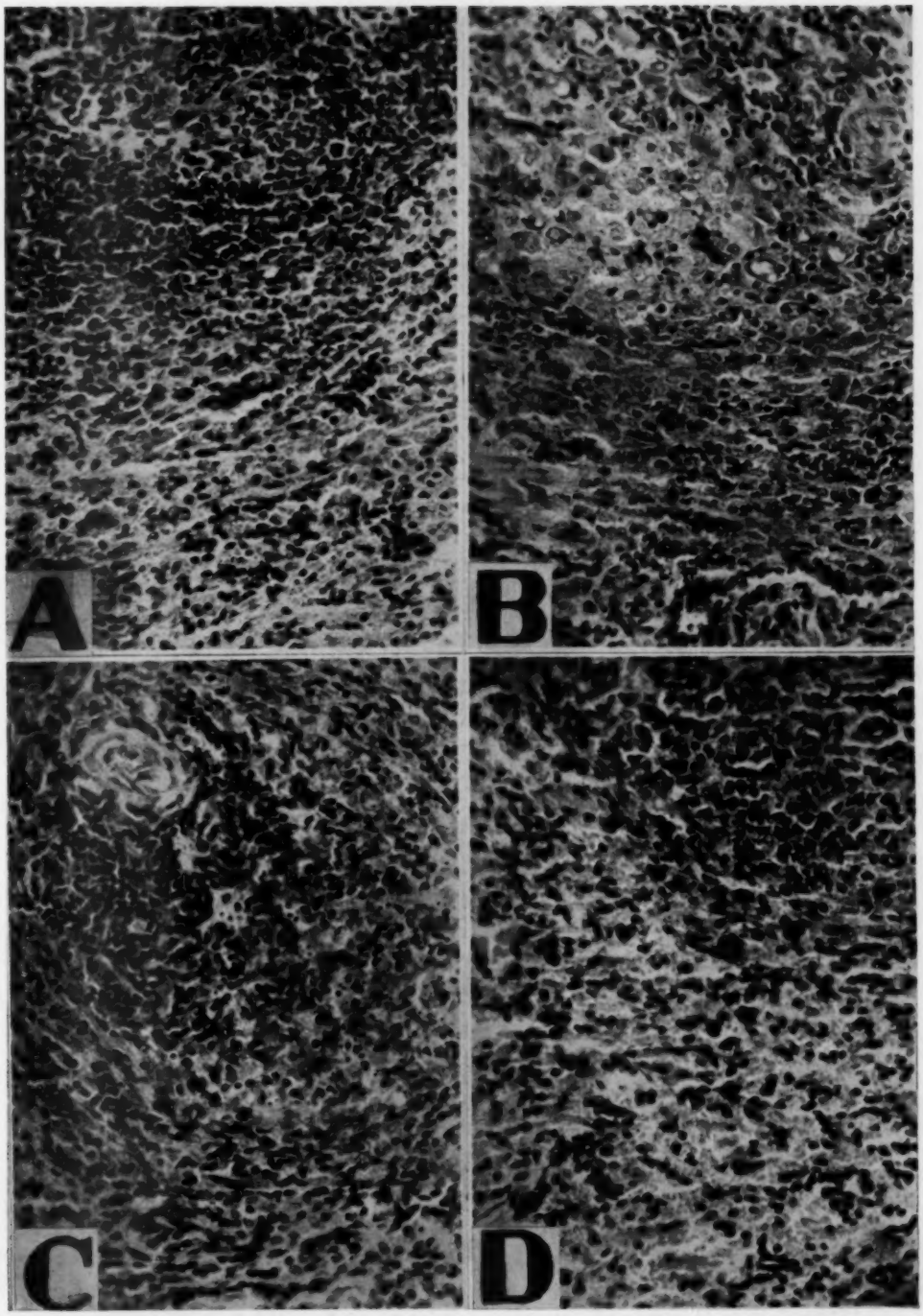

Fig. 1.—Spleens of ground squirrels. Azure A-eosin B stain; reduced about 2/5 from mag. × 475. *A*, normal nonhibernating ground squirrel. Section through the edge of a lymphoid follicle showing the marginal zone. *B*, one day after 800 r whole-body x-irradiation, nonhibernating ground squirrel. Necrotic cells and nuclear debris are prominent in the "reaction centers" and red pulp. *C*, one day after 800 r whole-body x-irradiation, hibernating ground squirrel. More nuclear debris is present than in the nonhibernating animal, but the extent of damage is nearly the same. *D*, 29 days after 800 r whole-body x-irradiation, hibernating ground squirrel. Less nuclear debris remains than at one day. There is no apparent regeneration.

29 days after 800 r whole-body x-irradiation given during hibernation with the animals maintained in the hibernating state after irradiation. Another group of three animals which had been maintained in deep hibernation for 29 days after irradiation was killed 3 days after awakening from deep hibernation.

Histological Observations of Normal Ground Squirrels.—The spleen has lymphoid tissue present both in a follicular pattern and as small nests of lymphocytes (Fig. 1*A*). Blood sinuses are prominent and appear to be lined by a single layer of cells. Ectopic erythropoiesis and granulopoiesis are inconspicuous. Hemosiderin is present in small amounts in sinusoidal macrophages. The lymph nodes resemble those seen in other rodents with lymphatic nodules within the denser lymphatic tissue of the cortex. The femoral bone marrow has an average cellularity of about 30% of the marrow area with adipose tissue making up the remainder (Fig. 2*A*). Erythroid elements are less abundant than myeloid elements. Mature granulocytes are numerous. Eosinophile leucocytes are apparently absent. Focal areas of hepatitis and focal granulomatous lesions are seen in occasional animals.

Histological Observations of Hibernating Ground Squirrels.—In spite of exsanguination the spleen shows acute passive congestion characterized by marked distention of the sinuses with erythrocytes. The follicular marginal zone, consisting of reticular cells and medium-sized lymphocytes, is smaller than normal. These changes disappear when the animal awakens from hibernation. The lymph nodes show no appreciable change. The bone marrow contains somewhat fewer mature granulocytes than normal, but there is no other significant change. The liver cords are narrower and the cells are smaller and less vacuolated in appearance than normal. Other organs show no change.

Histopathological Observations of Irradiated Nonhibernating Ground Squirrels.—At six hours after irradiation, there is prominent nuclear debris in the spleen, both in the lymphoid follicles and in the red pulp. The lymphoid follicles are smaller, and the follicular marginal zone surrounding the follicles is narrower, than normal. The lymph nodes contain prominent nuclear debris both in the follicles and diffusely in the cortex. The bone marrow also contains nuclear debris and shows some loss of hematopoietic cellularity and a decrease in mature granulocytes. Moderate hyperemia is present.

By one day after irradiation, atrophy of the lymphoid follicles in the spleen is marked. "Reaction centers" are prominent (Fig. 1*B*). The marginal zone of cells surrounding the follicles is virtually absent. Some nuclear debris remains in the atrophic follicles and in the red pulp. An increased quantity of hemosiderin is present in the red pulp. The lymph nodes also show marked atrophy with "reaction centers" in the lymphoid follicles. A moderate amount of nuclear debris remains in the follicles as well as in the outer cortex. The bone marrow cellularity is severely reduced, with an increase in numbers of megakaryocytes but nearly absent erythropoiesis and granulopoiesis (Fig. 2*B*). There are moderate numbers of mature granulocytes remaining, however. Moderate hyperemia is apparent.

At three days after irradiation, the appearance of the spleen is much the same as at one day. Some increase in ectopic myelopoiesis is evident. The lymph nodes show little further change. At all times after x-irradiation, the lymph nodes and spleen contain more small and medium-sized lymphocytes than are seen after comparable doses of irradiation given to rats.[6] The bone marrow is very atrophic; erythropoiesis and granulopoiesis are almost absent. Very few mature granulocytes remain, and congestion is marked. Megakaryocytes are slightly increased.

Histopathological Observations of Irradiated Hibernating Ground Squirrels.—At six hours after irradiation, nuclear debris is present both in the follicles and in the red pulp. The amount of debris, however, is less than that seen at the same time after irradiation of normal animals. Some atrophy of lymphoid follicles and decrease in cellularity

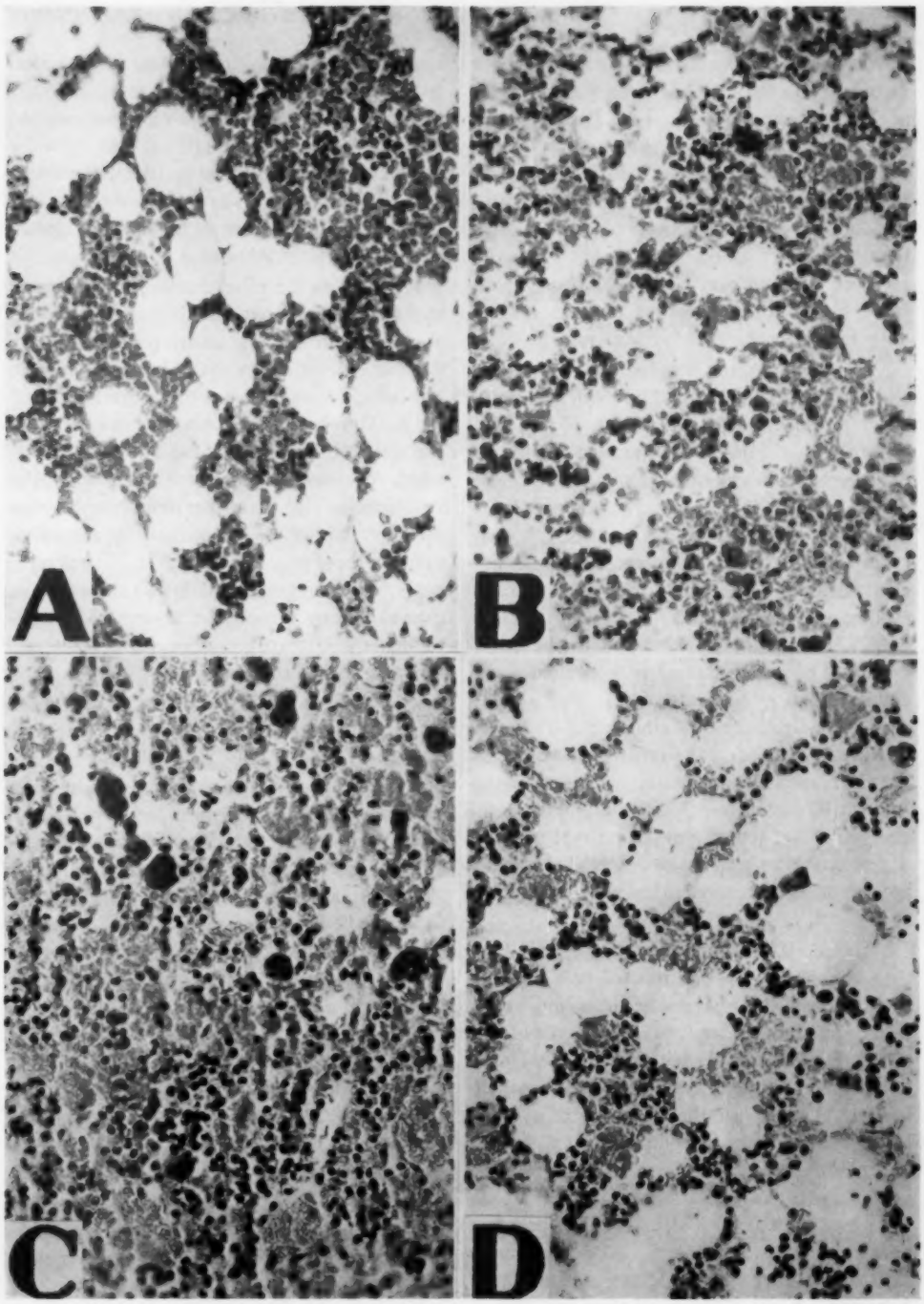

Fig. 2.—Bone marrow of ground squirrels. Azure A-eosin B stain; reduced 2/5 from mag. × 475. *A*, normal nonhibernating ground squirrel. Myeloid elements are more abundant than erythroid elements. *B*, one day after 800 r whole-body x-irradiation, nonhibernating ground squirrel. Granulopoiesis and erythropoiesis are almost absent, but megakaryocytes are increased. *C*, one day after 800 r whole-body x-irradiation, hibernating ground squirrel. Damage to the hematopoietic tissue is nearly the same as in the nonhibernating animal. Congestion is more marked, however. *D*, 29 days after 800 r whole-body x-irradiation, hibernating ground squirrel. Atrophy of the hematopoietic tissue is severe, but moderate numbers of mature granulocytes are present. No regeneration is apparent.

of the marginal zone around each follicle are already present. There is passive congestion of the splenic sinuses. The lymph nodes show atrophy and prominent nuclear debris. Here, also, the amount of debris appears to be less than that seen at the same time after irradiation of normal animals. The bone marrow shows only slight congestion and increase in megakaryocytes. Mature granulocytes are more numerous than in normal animals six hours after irradiation.

At one day after irradiation, atrophy of the lymphoid follicles of the spleen is severe; "reaction centers" are present (Fig. 1*C*). The marginal zone surrounding the follicles is absent. A moderate amount of nuclear debris remains both in the lymphoid follicles and in the red pulp. In contrast to the nonhibernating animals, no increase in hemosiderin is noted. In the lymph nodes there is moderate atrophy with prominent nuclear debris both in the follicles and in the outer cortex. The bone marrow is somewhat more cellular than in normal animals at a similar time after irradiation. Cellularity is reduced, however (Fig. 2*C*). Mature granulocytes are decreased in comparison with those seen in animals at six hours after irradiation and are somewhat fewer than in normal animals at one day after irradiation. Megakaryocytes appear to be increased. Moderate congestion is present.

At three days after irradiation, little further change is noted in the spleen. There is less debris in the atrophic follicles and in the red pulp than at one day, and congestion of the red pulp is still observed. Hemosiderin is not increased. The lymph nodes show little further change. In the bone marrow there is further decrease in cellularity with severe depression of erythropoiesis and granulopoiesis. Megakaryocytes are more numerous than normal. Nuclear debris is still present, and congestion is marked. Moderate numbers of mature granulocytes still remain, however.

At three days after irradiation, the hibernating animals show some preservation of mature granulocytes in the bone marrow and no increase of hemosiderin in the spleen. In normal animals at this same interval after irradiation there are very few mature granulocytes remaining in the bone marrow, and an increase in hemosiderin in the spleen is apparent. In other respects the hibernating and normal animals show the same type and nearly the same degree of histological change in response to irradiation.

At 29 days after irradiation, the spleen of the hibernating irradiated ground squirrel shows essentially the same picture as at 3 days after irradiation. Atrophy of the lymphoid follicles is somewhat more marked (Fig. 1*D*). Hemosiderin is not increased in the red pulp. In the lymph nodes there is somewhat less debris than at three days after irradiation. The bone marrow shows severe atrophy, which is somewhat more marked than at three days (Fig. 2*D*). Megakaryocytes are increased, and erythropoiesis and granulopoiesis are almost absent. Moderate numbers of mature granulocytes remain, and there is severe congestion.

Whole-body exposure of 800 r to normal ground squirrels causes 100% mortality within 30 days.[1] Thus, the hibernating animals killed at 29 days after irradiation show prolonged survival despite histological evidence of marked radiation damage. Regeneration was not seen in any of these animals. Mice, after similar doses of x-irradiation, show early evidence of regeneration at 13 days,[13] and several rodents show almost complete histological recovery at 4 weeks after irradiation with lower doses.[6]

At 3 days after awakening from 29 days of hibernation following irradiation, the spleen shows no further change except for somewhat less congestion and some decrease in nuclear debris. The lymph nodes show no change except for some decrease in nuclear debris. The bone marrow remains atrophic and congested. There is decrease in megakaryocytes, and mature granulocytes are almost absent.

COMMENT

There are indications that the primary reactions occurring after exposure to ionizing radiations are independent of body tempera-

ture over a wide range and are physical or photochemical in nature.[7] Frogs kept at low temperatures after x-irradiation exhibit greatly prolonged survival times, but there is no change in ultimate mortality.[2] The circulating leucocytes of such frogs decrease slowly after irradiation.[8] The hematopoietic tissue from frog tadpoles maintained at low temperatures after irradiation shows a very slow rate of cell destruction. The rate of break-down increases with increase in temperature.† Thus, the destructive effects of x-irradiation on frog hematopoietic cells appears to be dependent largely upon the environmental temperature after irradiation. There seems to be little if any recovery during the prolonged periods of survival at low temperature.

Hibernating mammals also demonstrate prolonged survival without decrease in ultimate mortality when maintained in the hibernating state after irradiation.‡ The reasons for this prolonged survival during hibernation have not been satisfactorily explained. Delay in development of histopathological changes does not seem to occur in hibernating mammals as it does in frogs. Smith and Grenan [10] noted that the bone marrow of irradiated hibernating woodchucks showed evidence of severe damage three weeks after irradiation. Brace [11] observed that irradiated, hibernating marmots had no active lymphoid, myeloid, or erythroid tissue. However, he reported that nonirradiated hibernating marmots showed a similar histological appearance, which suggests that the hematopoietic atrophy in this instance might have been on a nutritional or other basis.

The results of this investigation show that histological evidence of hematopoietic cellular damage following irradiation develops almost as rapidly in hibernating ground squirrels as in normal animals. The ultimate extent of damage appears to be almost the same in both groups. But there is some delay in removal of nuclear debris from the reticuloendothelial organs of hibernating animals when compared with nonhibernating animals. It would seem likely that cellular regeneration is greatly delayed in the blood-forming organs of the irradiated hibernating ground squirrels.

An interesting observation is that little or no hemosiderin accumulates in the spleens from irradiated ground squirrels maintained in hibernation. There is, however, moderate accumulation of hemosiderin in the spleens of nonhibernating ground squirrels after irradiation. Brace [12] noted an increased life span of erythrocytes in the hibernating marmot. Smith and Grenan [10] observed that anemia did not develop in woodchucks irradiated in hibernation until after hibernation was terminated. The anemia developing early after irradiation has several characteristics that suggest that it is hemolytic in nature.[13] Hemolytic anemia is associated with increased hemosiderin deposition in the spleen. It seems likely that acute anemia does not develop and hemosiderin does not increase in spleens of irradiated ground squirrels maintained in hibernation because the integrity of the erythrocyte and/or the capillary wall is maintained.

These studies do not offer definite evidence against the suggestion that development of cellular damage is dependent upon body temperature following irradiation. Although the body temperature of the hibernating ground squirrel remains nearly that of the environment, hibernation is not continuous.[5] Body temperature increases when an animal awakens. Intermittent awakening, accompanied by increase in body temperature, might be expected to hasten development of histological changes in an animal which remained in hibernation most of the time.

Survival is markedly prolonged in the ground squirrel by hibernation during and after irradiation, although histological damage occurs to about the same extent as in nonhibernating animals. Therefore, explanations other than cellular preservation in hematopoietic organs must be offered to explain the increased survival. Anemia and leucopenia apparently do not develop in the irradiated hibernating animal,[10] and this

† References 3 and 4.

‡ References 1 and 9.

study offers indirect support of this observation, since mature granulocytes were preserved in bone marrow and little hemosiderin appeared in the spleen when compared with nonhibernating ground squirrels.

Pathogenic bacterial growth is almost certainly inhibited by temperatures at which the hibernating animals are maintained. It seems likely that increased survival of hibernating ground squirrels after whole-body x-irradiation is related to reduction in infection and bacteremia due to suppression of bacterial growth possibly supported by absence of anemia and leucopenia rather than delay in development of cellular damage in the tissues.

SUMMARY

The histopathology of the hematopoietic tissue of the ground squirrel (Citellus tridecemlineatus) has been studied during hibernation at varying times after 800 r whole-body x-irradiation, and at varying times after 800 r whole-body x-irradiation given to animals maintained in the hibernating state during and after irradiation. The rate of development of cellular damage is nearly the same in nonhibernating and hibernating animals. The extent of damage is identical in both groups. Cellular regeneration in the hematopoietic tissue is delayed in the hibernating animal. Indirect evidence of preservation of mature leucocytes and erythrocytes after whole-body x-irradiation of the hibernating ground squirrel is offered. The increased survival time, as a result of hibernation, appears to be due to other factors than alteration in development of cellular damage.

Technical assistance in this study was given by Miss Audrey Cliffgard. The photomicrographs were taken by Mr. Jean Crunelle, Chief Photographer, University of Chicago Clinics.

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Observations on the Intraperitoneal Injection of Aluminum Hydroxide in Mice

SAUL KAY, M.D.
and
JOHN L. THORNTON, M.D., Richmond, Va.

This experimental study is an outgrowth of clinical and experimental experience with barium sulfate contrast medium.* Results of injection of barium into the peritoneal cavity of mice have been published previously.[3] It was seen that the basic response was a histiocytic and fibrous granuloma which was essentially nondeleterious to the host.

Since aluminum hydroxide preparations are widely used in the treatment of peptic ulcers, it would theoretically be possible for these compounds to gain access to the peritoneal cavity as a result of penetrating or perforated ulcers. While, in the case of perforated ulcers, the resulting chemical peritonitis would be expected to mask any effect which aluminum alone may produce, it is postulated that there is a definite host reaction to this element. To determine the nature of this reaction, the animal experiments were undertaken.

A review of the literature shows that German investigators have been aware of soft-tissue reactions to aluminum hydroxide. For example, Beck [4] in 1954 described the formation of granulomas in the subcutaneous tissue following immunization against tetanus. The material used was Tetanol, which consists of tetanus toxoid adsorbed on aluminum hydroxide. Among 37,000 inoculations, 7 patients developed localized nodules which were excised and studied histologically, bacteriologically, and chemically. Pathologic examination showed central cavities lined by layers of epithelioid cells. In the center of the cavities was found amorphous lumpy material ultimately identified as aluminum hydroxide. Some of this material was associated with foreign-body-type giant cells. Small tubercles were noted adjacent to the excavated areas. The intervening stroma was densely infiltrated with lymphocytes and polymorphonuclear leucocytes.

Fig. 1.—Appearance of peritoneal cavity 60 days after the intraperitoneal injection of 0.5 cc. of aluminum hydroxide. Note pools of white material covering various viscera.

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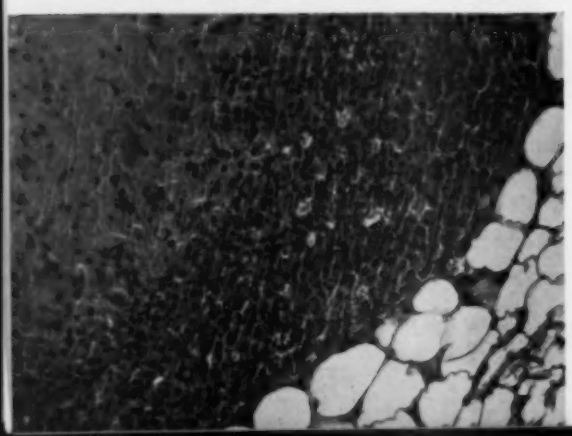
From the Department of Surgical Pathology, Medical College of Virginia.

* References 1 through 3.

Fig. 2.—Low-power view of pool of aluminum hydroxide within fat. Note collections of histiocytes at periphery; 60 days after injection of 0.1 cc. of aluminum.

Observations in animals were contributed by Cohrs and Schulte in 1952.[5] The authors noted the development of subcutaneous granulomas in cattle following injections of various vaccines adsorbed on finely divided aluminum hydroxide. Essentially an encapsulated lesion was produced, composed of several cavities separated by fibrous septa. The chambers were filled with caseous material surrounded by granulation tissue. Between the fibrous septa and foreign material there were numerous histiocytes, larger than ordinary macrophages and resembling squamous cells. The authors considered these cells pathognomonic for granulomas due to aluminum. A few giant cells of the foreign-body and Langhans type were also noted. Within some of the giant cells, and also lying freely between cells, were needle-like crystals identified histochemically as aluminum hydroxide. Similar granulomas could be produced in calves and guinea pigs by the injection of pure aluminum hydroxide. Incidentally, the regional lymph nodes were always found to be free of any involvement.

Fig. 3.—High-power view of Figure 2 to show details of histiocytic infiltration.



MATERIAL AND METHODS

In general, methods similar to those described in the previous report were used.[3] A total of 18 Swiss albino female mice were utilized, 3 to serve as controls. They were approximately 5 months old, with an average weight of 34 gm. The proprietary aluminum hydroxide preparation "Creamalin," which to all intents and purposes is a pure suspension of aluminum hydroxide, was utilized for injection purposes. Since this material is bacteriologically sterile and is of low viscosity, it was found to be ideal for the purposes of this experiment. Fifteen mice were divided into groups of three, and each group received 0.1, 0.2, 0.3, 0.4, and 0.5 cc., respectively. The aluminum was injected intraperitoneally with sterile precautions. It was originally planned to kill each group at two-month intervals, thus carrying our observations up to a six-month period. Unfortunately, many mice unaccountably disappeared from their cages, allowing for a maximum observation period of only four months. No mice died as a result of the injections, and they were killed at two- and four-month intervals, each interval representing an entire group of animals injected with all the varying doses.

RESULTS

After 60 days small localized collections of aluminum hydroxide could easily be found within the peritoneal cavity. These foci varied from 1 to 6 mm. in diameter and were larger with the greater doses employed (Fig. 1). The location of the aluminum pools was apparently haphazard and probably varied with the movements of the animal. For example, localized pools were found on the surface of ovaries, colon, stomach, liver, mesentery, spleen, anterior peritoneum, and diaphragm. They were also found free in the peritoneal cavity. In addition, adhesions between viscera were noted, and these were more extensive with the larger doses. In the 0.5 cc. mouse, there were adhesions between the ileum and pelvic organs, between the liver and diaphragm, and between the spleen, stomach, colon, and liver.

Microscopically, aluminum hydroxide was fairly easily identified in the tissues, especially when localized pools were formed. Aluminum hydroxide is not doubly refractile, but in hematoxylin and eosin preparations it appears as amorphous, granular, and lightly basophilic material. With the smallest dose

employed, there were pools in the anterior abdominal wall and fat (Figs. 2 and 3). These pools were surrounded by mononuclear phagocytes which appeared to engulf the material. There was minimal nuclear fragmentation and minimal fibrosis. No tubercle formation was present. With a 0.2 cc. dose a distinct fibrous reaction could be discerned. There were, for example, adhesions covering the liver surface and between the liver and diaphragm (Fig. 4). In addition, tiny pools of aluminum could be found within the liver substance. These may have been transported by means of the reticuloendothelial system. As the dose was increased, the fibrous reaction became severer and necrosis of phagocytes was observed. This was associated with a polymorphonuclear leucocytic infiltration. Adhesions between viscera were more plentiful, and there was perivisceral as well as perinodal fibrosis.

In summary, the basic response was a fibrous and histiocytic granuloma, without tubercle formation or giant cells. The adhesions were localized and apparently caused no obstructive phenomena, since no intestinal distention was present.

The mice killed 120 days after injection showed similar quantitative changes dependent on the dose employed. The 0.4 cc. and 0.5 cc. mice had milky peritoneal fluid, but this was apparently not of bacterial origin, as all organs showed a smooth and glistening surface and fibrin was absent. Worthy of mention were the large white plaques seen on the surface of the liver.

Microscopically more fibrous and histiocytic reactions were seen than in the first group of mice. Aluminum-containing histiocytes were more readily observed in the liver. The fibrous reaction was maturer and often hyalinized (Fig. 5). Adhesions were thicker and more prominent, and many of the viscera showed a subserous fibrous reaction (perisplenitis, perihepatitis, etc.). There was considerably more nuclear fragmentation and polymorphonuclear leucocytic infiltration in some of the granulomas (Fig. 6).

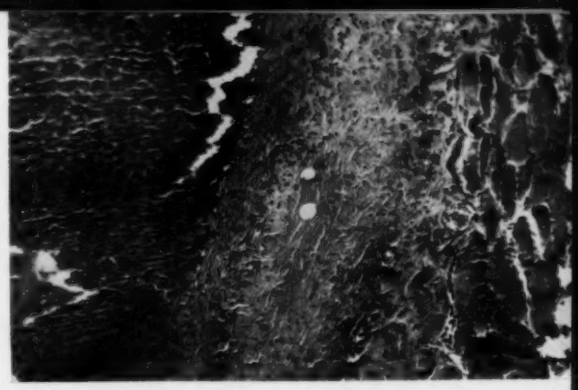


Fig. 4.—Fibrous adhesion between liver and diaphragm; 60 days after injection of 0.2 cc. of aluminum.

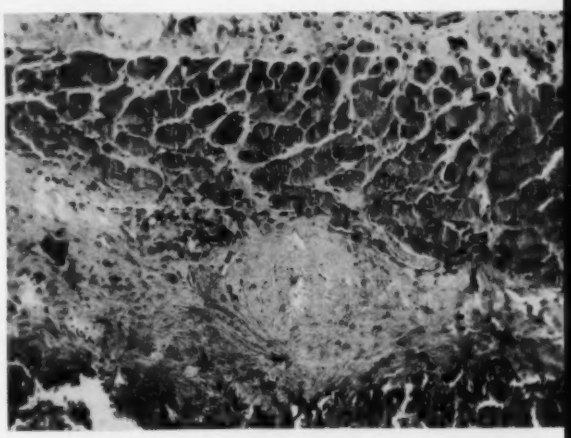
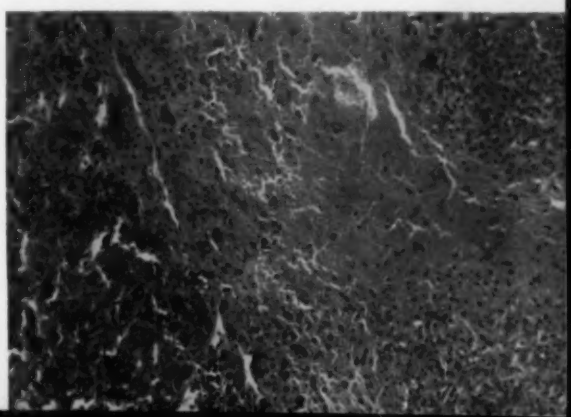


Fig. 5.—Adhesions between liver and diaphragm; 120 days after injection of 0.2 cc. of aluminum.

In summary, the older animals showed reactions similar to those of the younger, but the fibrosis was severer and maturer. Adhesions were tougher, and subcapsular fibrosis of the viscera was a prominent feature. Tubercle formation and giant cells were absent. Again, obstructive phenomena were not observed

Fig. 6.—Marked perihepatic reaction to aluminum. Note nuclear fragmentation and infiltration with histiocytes and a few polymorphonuclear leucocytes; 120 days after injection of 0.5 cc. of aluminum.



COMMENT

Apparently the tissue response to aluminum hydroxide is similar to the response to barium sulfate. The basic reaction is a histiocytic granuloma associated with fibrosis which becomes thicker and maturer with increased dosage and more prolonged observation. While adhesions are produced, these do not become severe enough to cause intestinal distention or obstruction. With barium some intestinal distention may be expected with larger doses.

No deleterious effect caused by the aluminum injections could be noted in the mice, at least for the four-month observation period, as herein described, which is approximately one-sixth the normal life-expectancy of the animal.

Concerning the doses of the injected material employed, the effect of 0.1 cc. of aluminum hydroxide would be equivalent to about 200 cc. in man. The latter dose would be an unusually large amount expected to reside in the human stomach at any one time. Consequently, the result of a perforated ulcer in a patient using aluminum hydroxide gel therapy would be due to the ensuing peritonitis rather than to any reaction the aluminum could produce. Nor is there any proof that the leakage of aluminum into the peritoneal cavity would necessarily affect the mortality or morbidity of the individual ulcer patient concerned.

SUMMARY

Injection of aluminum hydroxide into the peritoneal cavity of mice produces a histiocytic and fibrous granuloma. The latter causes adhesions between viscera and pelvic organs, but obstructive phenomena do not result from the reaction.

No deleterious effects upon the animals in the four-month observation period employed were noted.

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Dystrophic Calcification in the Adrenal Glands of Monkeys, Cats, and Dogs

LIEUT. COL. MARTIN A. ROSS (VC)
CAPT. J. H. GAINER (VC)
and
J. R. M. INNES, Sc.D., Ph.D.
Army Chemical Center, Md.

In any program of research in which thousands of laboratory animals are brought to autopsy annually, most of the common specific diseases will be encountered. Of equal interest, however, will be the incidental finding of lesions of diverse natures, causes, and anatomical distribution, which manifestly might have been clinically silent, and which might have been irrelevant to the result of the experiment concerned. A long list of lesions of the latter category could be compiled from our experience since 1952, during which time autopsies have been done on approximately 15,000 animals by the staff of our department. The present paper is one of a series of studies, viz., on muscular dystrophy in rabbits,[1] pulmonary acariasis in monkeys,[2] chronic pulmonary disease in rats,[3] inherited dysplasia of the hip and shoulder joints of rabbits,[4] septic phlebothrombosis caused by Salmonella in hamsters,[5] and sarcosporidiosis.[6]

We need not delve into the extant literature on calcification including the dystrophic variety, because it is succinctly covered in an admirable chapter by Cameron[7] (1951) on "The Pathology of the Cell." We are still far from good understanding of the complexity of physiological calcification, not to mention the intricate mechanisms underlying dystrophic processes. Calcification has been known for a hundred years to occur commonly in devitalized or necrotic tissues, but this is not a universal sequence, and there are differences due to the species involved.

Calcareous concretions were first observed in the adrenal glands of a monkey, which stimulated the need for further data, but the same lesion had been shown to one of us (J. R. M. I.) by Major C. Barron (VC), in the Armed Forces Institute of Pathology, Washington, D. C., in 1951. We have been unable to find any references to observations on monkeys, but mention of calcification in feline adrenal glands can be traced back in German literature to Joest[8] (1923), as discussed later.

MATERIAL AND METHODS

Reports on autopsies since 1952 on cats, monkeys, and dogs, and related sections were reexamined. Complementary to this action a start was made to collect both adrenal glands as part of postmortem examination technique. The animals either had been exposed experimentally to a variety of toxic chemical compounds or had died naturally. Sometimes only one adrenal gland was available, but in the routine collection both were studied. The total examination embraced glands from 54 monkeys (Macacus mulattus), 46 cats, and 57 dogs. Tissues were fixed in 10% formalin saline, embedded in paraffin, and stained with hematoxylin-eosin; special tests for calcium (alizarin red), for iron (Perls' test), and for other materials were done on selected tissues. Through the courtesy of Dr. Ross McCardle, National Cancer Institute, Bethesda, Md., a sample series of sections were microincinerated.

RESULTS

Calcareous depositions were found in the adrenal glands of 27 out of the 54 monkeys,

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Pathology Branch, Chemical Corps Medical Laboratories, Army Chemical Center, Md.

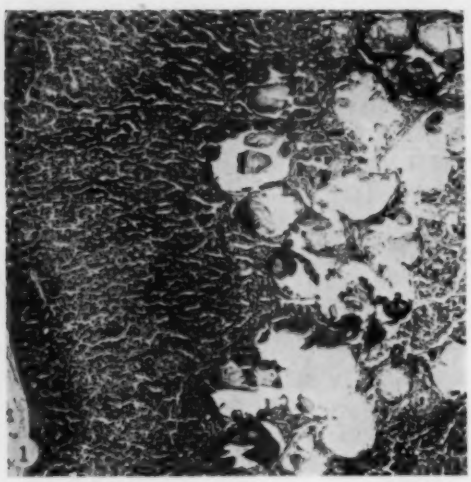
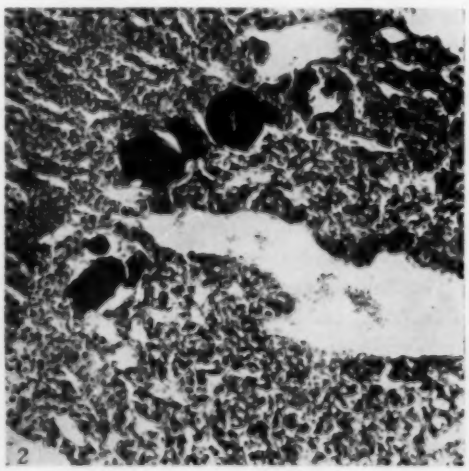


Fig. 1.—Adrenal, monkey. Calcareous deposition in corticomedullary region; much crumbling and falling-out in the section, leaving irregular holes. Hematoxylin-eosin; reduced about ⅓ from mag. × 80 (463.54B).

14 of the 46 cats, and 2 of the 57 dogs.* There was no clear relationship to sex, and it was not a biological phenomenon of advancing age, for most of the cats, dogs, and monkeys used in our laboratories are mature, but young, animals. It is uncertain whether the lesion always occurs bilaterally; in many animals only a single adrenal gland was available for examination, and because of restriction in mass and location of the lesion it might be missed on chance sectioning of any one gland. Because of the variety of experimental procedures (in the sense of chemical agents employed), the lesion could not have been caused by any particular chemical substance. There were differences in the nature of the lesion of the three species.

In the monkeys, without exception, the calcifications were found restricted to the corticomedullary region, and more frequently in the zona reticularis than in the medulla. They were not found in the zona fasciculata and zona glomerulosa, or in the capsule, vessels, nerves, or ganglia outside the capsule. The calcifications were roughly round in shape, varying in size (up to 100μ to 150μ) and in number; there were only a few in some monkeys, but in others there were a great number scattered around without sign of confluence. There was a tendency for crumbling and a falling-out of the mineral deposits from the sections, so that in the severe cases the corticomedullary region was riddled with irregular holes (Fig. 1). Some cases showed that possibly as much as 30% of suprarenal tissue had been destroyed. Intact bodies were lamellated and were manifestly acellular (Fig. 2). With hematoxylin-eosin the staining propensity varied; some were deeply basophilic, others were a dark rust-brown, and yet others were a watery-pink. The deposits stained red to deep purplish-black by alizarin red (Fig. 3), which, as far as we know, is specific for calcium salts, and they were impregnated black by silver salts (von Kossa's reaction). The bodies did not react positively in tests for the presence of iron, although small amounts were demonstrable by microincineration. Inflammatory reaction had not

Fig. 2.—Adrenal, monkey. Another case showing discrete lamellated bodies with no surrounding reaction. Hematoxylin-eosin; reduced about ⅓ from mag. × 167 (596.54A).



* Since the study was completed, we have been informed that calcification in the adrenal glands has been observed by others; Dr. Leon Z. Saunders, Brookhaven National Laboratory, N. Y., found it in 4 cats, and Dr. R. Haberman, Section of Comparative Pathology, National Institutes of Health, Bethesda, Md., has noted it in 16 monkeys.

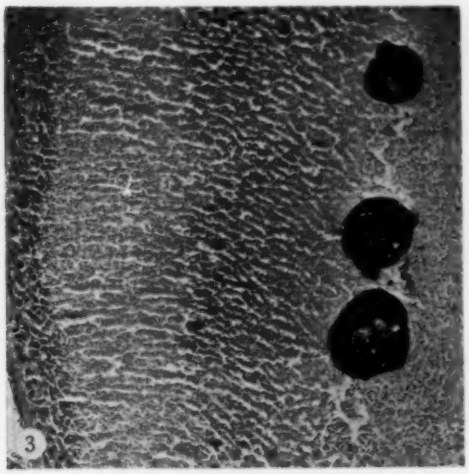
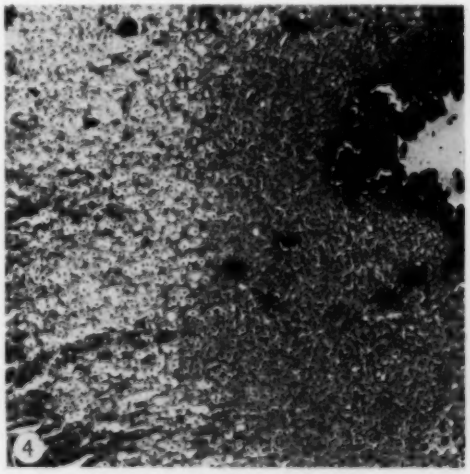


Fig. 3.—Adrenal, dog. Calcified bodies which stained a deep purplish-red by alizarin red; reduced about ⅓ from mag. × 167 (377.54).

been evoked, but each body seemed to be enclosed by a delicate capsule; yet, the latter could have been simply condensation of normal adjacent cells and reticulum squeezed by the accumulating deposition of minerals. There was no evidence of a preexisting necrosis, or other tissue abnormality such as amyloid or mucoid degeneration. In one case there was some concomitant hemosiderosis. As in most monkeys a great range of organs had been taken for histologic study, we can state precisely that the adrenal calcification was not part of a metastatic calcareous process. The deduction could not be made that it was caused by whatever experimental chemical trauma had been inflicted upon the monkeys, because the same lesion was found in animals dying before senility from natural diseases. The calcification had not enmeshed dead helminths or other parasites. The calcareous bodies could not be clearly identified by macroscopic examination alone, except in a few instances, although grittiness could be felt on cutting the fixed tissues. There was no tendency for the calcification to become vascularized and to proceed to ossification. All the above descriptive facts are applicable to the two canine cases, but differences existed in the case of the cat adrenal glands.

In three cats the same type of what might be called corpora calcificans was found. In addition, we were impressed by the sweeping variation in the general morphology of the feline adrenals, few glands from any two cats being strictly comparable. Apart from qualitative cell changes of the cortex, there were quantitative differences in the relative thickness of cortex and medulla, and of the three cortical layers. The arcuate character of the zona glomerulosa was pronounced in some glands, but it was lost or the zone was atrophic in others. From the highly vacuolated appearance of the cells in the cortex (in paraffin sections), the lipid content must have been abnormal (Figs. 4-8), and in some glands the appearance of the zona fasciculata and glomerulosa was reminiscent of advanced hepatic fatty metamorphosis. In glands showing the latter feature, another form of calcification was found as diffuse granular foci, which were sometimes extensive. In hematoxylin-eosin-stained sections the mineral deposits were basophilic and gave a positive reaction for calcium with alizarin and von Kossa's test, and a negative one for iron; again small amounts of the latter were demonstrable by microincineration. Although there was no clear evidence

Fig. 4.—Adrenal, cat. Massive granular deposits in corticomedullary region and scattered smaller areas in cortex; lipid degeneration in zona fasciculata. Hematoxylin-eosin; reduced about ⅓ from mag. × 80 (601.54B).



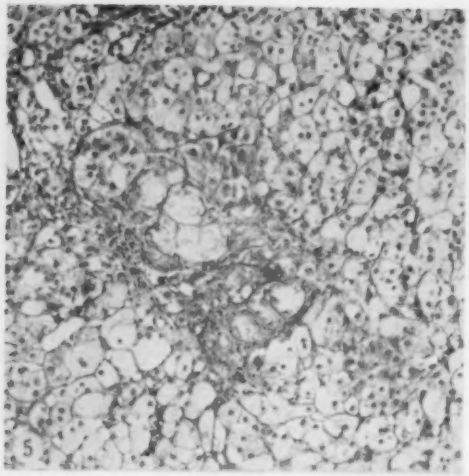


Fig. 5.—Adrenal, cat. Atrophy of the zona glomerulosa and severe vacuolated changes of rest of cortex and with basophilic granular deposits. Hematoxylin-eosin; reduced about ⅓ from mag. × 167 (545.54H).

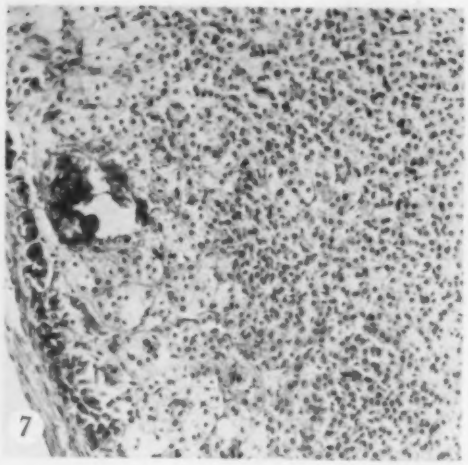


Fig. 7.—Adrenal, cat. Atrophy of zona glomerulosa; numerous discrete areas of calcification such as shown here were present in the cortex. Hematoxylin and eosin; reduced about ⅓ from mag. × 167 (545-54P).

of changes which might have been the precursor to the calcification, and no inflammatory reaction, the adrenal glands may have been abnormal, if the intense cellular vacuolation in cortical zones portended lipid degeneration. Like the monkeys, the cats had all been exposed to a variety of experimental procedures or had died naturally.

A few observations by Dr. Ross McCardle on the microincineration of some adrenals with calcification are appropriate. The ash residue of the calcified bodies consisted mainly of calcium, magnesium, silica, and a little iron. In some sections of the cat adrenal glands, compared with adjacent preparations stained by Masson's trichrome method, the

Fig. 6.—Adrenal, cat. Another case showing severe lipid change in zona fasciculata and irregular spaces due to falling-out of calcareous masses. Hematoxylin-eosin; reduced about ⅓ from mag. × 80 (545.541).

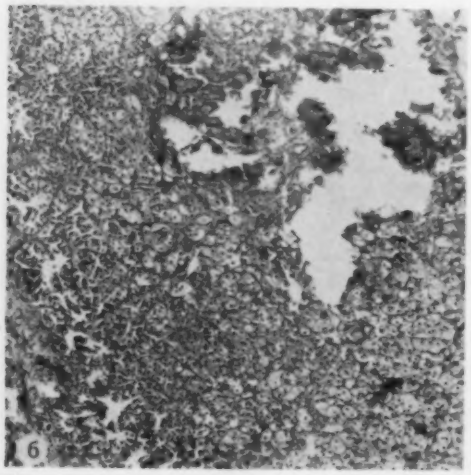
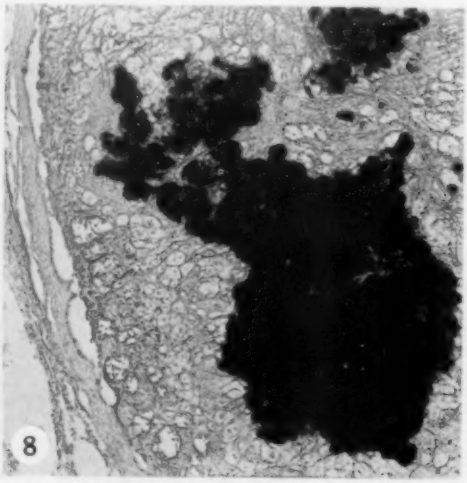


Fig. 8.—Adrenal, cat. A severely changed adrenal, with atrophy of cortex, lipid degeneration, and massive calcareous deposition brought out by alizarin red; reduced about ⅓ from mag. × 80 (6.46).



calcareous deposits occurred in a mass of vacuolated foamy degenerative cortical cells. Compared with the silver preparations the deposits were not uniformly blackened, and internal areas, not at all. The ash consisted of the residues of calcium and/or magnesium, silica, potassium, sodium, and very small amount of iron oxide, representing perhaps masked iron, and not easily demonstrable by the Prussian blue reaction. Dr. McCardle considered that the first stage in the process in cats might be tuberous projection of masses of cortical cells into the medulla, which became enlarged and laden with lipids and with later deposition of minerals on the cell surfaces and intercellular spaces.

COMMENT

The relatively frequent incidence of the calcification is of interest, even although, except in some cats, the lesion could not be regarded as severe enough to cause clinical disturbance. It is still a pathologic phenomenon requiring explanation. Basically, of course, we run up against incomplete understanding of the mechanisms involved in any form of pathologic calcification, which is readily apparent from the analysis by Cameron [7] (1951).

Two forms of calcification must be distinguished, regarding appearance, zonal distribution in the adrenal glands, and perhaps etiology. In all the three animal species, the type called corpora calcificans stood apart from that defined as granular in nature. In monkeys, cats, and dogs, the calcified bodies were found in the zona reticularis, and less commonly in the medulla. Only in the cat were the granular deposits seen in the cortex as well as in the medulla. In neither form, contrary to a common finding in so-called calcification, was there any definite associated deposition of iron salts as evinced by application of Perls' test, although minute amounts were demonstrable in microincinerated section.

There can be no dispute that the occurrence of the lamellated calcified bodies is neither part of a metastatic calcification nor a sequel to a preexisting identifiable change, such as necrosis or amyloid or mucoid degeneration. The bodies apparently had arisen in an intercellular situation, gradually increased in size by successive layerings of mineral deposit, and did not enclose cells, foreign material, or parasites. In that sense, they are no different from the classical so-called corpora amylacea, which in different animals and varied anatomical locations are not amyloid in nature at all. Their presence and distribution in human tissues have been known since Virchow coined the name, and the anatomical sites mentioned as being commonly involved do not usually include adrenal glands. In domesticated animals, some of the well-known locations for corpora amylacea include the udder of the cow (Fig. 9), the prostate of the dog, and the dura mater of the horse and the dog (Nieberle and Cohrs,[9] 1952), but not the adrenal gland. (In the canine dura mater, there is a tendency for the bodies to become ossified, which may well be the basis for a condition developing in old dogs and known as chronic ossifying pachymeningitis). We know just as little about the cause and pathogenesis of corpora amylacea in domestic animals as in man, although in the udder and prostate the concretions must be associated with disturb-

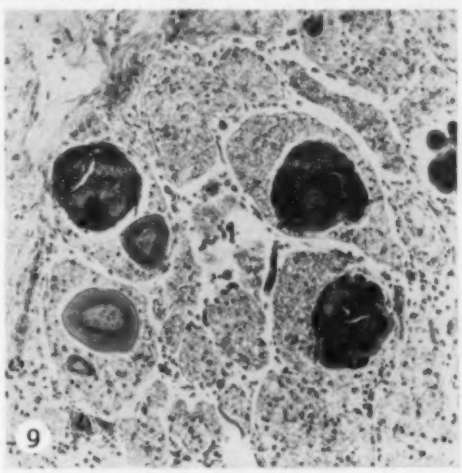


Fig. 9.—Udder, cow. Corpora amylacea. Typical appearance of common condition in the udder; innumerable lamellated bodies present all through the section. Hematoxylin-eosin; reduced about ⅓ from mag. × 167 (215/27).

ances in glandular secretion, for they mostly reside in the glandular lumina.

In many instances, as we range through mammalian pathology, calcification is a postnecrotic process; but this is not a constant sequel, and the differences are curious. For example, necrosis and caseation in tuberculosis of both the dog and monkey is the rule (Fig. 10) (Innes,[10] 1942), but calcification, in contrast to tuberculous lesions of cows and man, is the exception. In dogs, on the other hand, (*a*) the calcification proceeding to ossification of benign and malignant mammary tumors is notorious, (*b*) calcification

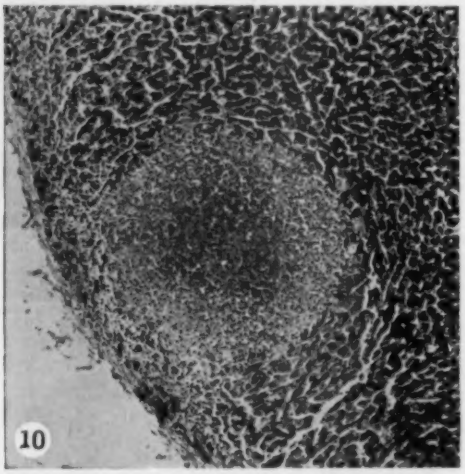


Fig. 10.—Adrenal, monkey. Tuberculosis. Adrenal involved in acute miliary dissemination; tubercle with central caseation and no calcification. Hematoxylin-eosin; reduced about ⅓ from mag. × 80 (6.46).

without previous necrosis is common in the aortic tunica media of old animals, and (*c*) calcification is frequent in the kidneys (Bloom,[11] 1955). In all such tissues involved there is normally a high alkaline phosphatase content which we know plays a vital role in calcification and ossification, normal and abnormal (Cameron [7]).

In the case of the cat, observations on adrenal calcification date back to Joest [8] (1923). Five original works were also found, viz., Brüschweiler [12] (1924), Fuhrer [13] (1925), Marine [14] (1926), von Bögli [15] (1928), and Drieux and Thiery [16] (1943). The findings of all are in harmony regarding the relative frequency of adrenal calcification and its occurrence in young cats under 6 months old. Bögli also drew attention to the morphologic variation in the "normal" cat adrenal (see also Nicander,[17] 1952) and observed that calcification might be a postnecrotic sequel in some, or follow extreme lipid degeneration of the cortex in others. Calcification of thrombi was considered as one explanation, but we did not find that either form (bodies or granular) of calcification occurred in arteries, veins, or sinusoids. The divergent morphologic status of so-called "normal" cat adrenals and the palpable susceptibility of the gland to pathologic change indicate that the feline adrenal must be a highly labile one, and perhaps more prone to disease than veterinary clinical literature indicates.

We do not know whether there is any substance in a suggestion, by Brüschweiler,[12] that in cats the lesion is caused by a disturbance in calcium metabolism initiated by chronic enteritis. Hypercalcemia alone was proved very simply by experiment not to result necessarily in calcification of the adrenals. For example, metastatic calcification with its concomitant hypercalcemia and high calcium urinary excretion in hypervitaminosis D is an old story, and in rats and rabbits the tissues commonly affected are aorta, coronary arteries, kidneys, and smooth muscle, but not the adrenal (Harris and Innes,[18] 1931). Two experimental cats were nevertheless injected with 500,000 international units of activated ergosterol daily for 14 days; the serum calcium rose from around 10 to 16 mg. per 100 cc., and the animals lost appetite and weight and were then killed; massive calcific lesions were found in the usual sites, being of devastating severity in the aorta, but both adrenal glands were free from calcification. The adrenal calcification is thus not a form of natural vitamin D toxicity. Marine [14] found the calcareous deposits (and from his pictures they resemble the lesions described by us) in the adrenals of 64 out of 257 cats, but not once in the adrenals of

328 dogs, 2500 rabbits, 150 pigs, 200 sheep, and 125 cattle. He also made chemical estimations of affected glands, and one severely affected adrenal, weighing 92.2 mg. (dry weight), contained 8.68 mg. of calcium, a notable amount. Marine concluded that it was a sequel to feline distemper infection (not known at that time to be caused by a virus), and that it occurred two to three weeks after the disease was first clinically manifest. We have been unable to associate any definitive clinical picture with the lesion, but few of our cats were seen alive. Marine stated that adrenal calcification was to be suspected if the cats failed to improve after subsidence of the initial illness, lost weight, and became weak and ataxic and that the listed signs resembled those in cats surviving two to three weeks after double suprarenalectomy. This is a trenchant statement and confined by the studies of Drieux and Thiery,[16] who found the lesion in 44 out of 50 cachectic cats and only in 6 out of 77 apparently healthy animals. There is some indication, therefore, that the lesion could be more than an incidental irrelevant one, as far as the clinical status of the cat is concerned, and the problem might thus be of practical importance in consideration of the pathology of the feline virus disease and when cats of unknown history are used for experiments. From the various observations cited, it is possible there may be a common clinical syndrome in cats, which has remained unidentified or masked by some other diagnosis. The fact that adrenal lesions have not been described in the literature on feline distemper may only signify that the glands have not been studied.

Consideration was given to the possibility that the corpora amylacea (or better, calcificans) in the three species might be concretions arising in the medullated nerve fibers (myelin sheath or axon) of the adrenals rather analogous to that seen in the human brain and cord (Dublin,[19] 1954). A study of serial sections of two adrenals proved that the bodies were isolated from each other and with no evident connection to any nerve (or artery or vein).

SUMMARY

Calcification was found frequently in the adrenal glands of 27 monkeys, 14 cats, and 2 out of 57 dogs. The common lesion in all monkeys, some cats, and the two dogs, was lamellated bodies, identical with those referred to as corpora amylacea in other tissues of other species, including man. The calcified bodies were restricted to the zona reticularis and medulla and had no precursor in an obvious form, such as necrosis or amyloid and mucoid degeneration; nor did they evoke inflammation. The granular form in the feline adrenal glands involved the cortex or medulla and might have been associated with a preexisting lipid metamorphosis or patchy necrosis. Neither form was associated with so-called metastatic calcification. The cause and pathogenesis is unknown, but the suggested role in the pathogenesis of feline distemper (by Marine) warrants some consideration, for the lesion might be responsible for a clinical picture hitherto unidentified.

Mr. John Cuculis, of the Pathology Branch, did the photography, and Dr. Ross McCardle, National Cancer Institute, Bethesda, Md., made and examined the microincineration specimens.

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News and Comment

ANNOUNCEMENTS

A. M. A. Annual Meeting, Chicago, June 11-15, 1956.—The 105th Annual Meeting of the American Medical Association will be held in Chicago, June 11-15, 1956, with a full program of lectures, television, motion pictures, and scientific exhibits.

The lecture program for each Section is prepared by the Section Secretary, and prospective participants should communicate as soon as possible with the Secretary of the Section before which they hope to read a paper. Application blanks for space in the Scientific Exhibit are now available and may be obtained from the Section Representative to the Scientific Exhibit.

Section on Pathology and Physiology

Secretary: Edwin F. Hirsch, M.D., 1439 S. Michigan Ave., Chicago 5.

Exhibit Representative: Frank B. Queen, M.D., 3181 S.W. Sam Jackson Park Rd., Portland 1, Ore.

Additional information may be obtained from the Secretary, Council on Scientific Assembly, American Medical Association, 535 N. Dearborn St., Chicago 10.

International Congress of Human Genetics.—The First International Congress of Human Genetics will be held in Copenhagen, Denmark, Aug. 1-6, 1956. This Congress is planned to cover all genetic aspects of normal and pathological characters in man. Any person interested in the subject of human genetics and especially of medical genetics is invited to take part in the Congress.

A provisional program and further information will be sent on request. Address inquiries to The Secretariate of the First International Congress of Human Genetics, The University Institute for Human Genetics, 14, Tagensvej, Copenhagen, N., Denmark.

Annual Symposium on Fundamental Cancer Research.—The 10th Annual Symposium on Fundamental Cancer Research will be held on March 29, 30, and 31, 1956, at the University of Texas M. D. Anderson Hospital and Tumor Institute, in the Texas Medical Center, Houston.

A panel discussion on "Nucleic Acid Metabolism in Tumors" will be under the direction of Dr. Van R. Potter, Professor of Oncology at the University of Wisconsin Medical School. Dr. Darrell Ward, Department of Biochemistry, M. D. Anderson Hospital, is in charge of the reports on recent developments in cancer research. Dr. Grant Taylor, Dean of the University of Texas Postgraduate School of Medicine, is general chairman of the symposium.

Malignant Granular-Cell Myoblastoma

LUCITO G. GAMBOA, M.D., Denver

True malignant granular-cell myoblastomas are rare. There are only 10 authentic examples in the world literature (Tables 1 and 2), although no less than 32 cases have been reported as such. The tumors reported by von Meyenburg,[8] Cioni,[9] and Loi [10]; in Cases 1, 3, 5, 6, 7, and 9 of Howe and Warren [11]; in Cases 2 and 3 of Khanolkar,[12] and in Cases 8, 9, and 10 of Murphy, Dockerty, and Broders [13] were probably rhabdomyosarcomas. Those recorded by Horn and Stout,[14] Hartz,[15] Ackerman and Phelps,[16] and in Cases 1, 4, 5, and 6 of Khanolkar [12] strongly resembled the morphological and clinical characteristics of what have been recently reported as malignant nonchromaffin paragangliomas [17] and alveolar soft-part sarcomas.[18]

REPORT OF A CASE

A Negro woman, 30 years of age, was admitted to the Corwin Hospital, in Pueblo, on July 6, 1949, complaining of a baseball-sized, hard, lobulated mass in the anterolateral aspect of the distal end of the right thigh and enlarged, tender lymph nodes in the right groin. She first noticed a small lump in the distal end of the right thigh in 1942. This gradually increased in size, and, from May, 1949, she experienced pain when the tumor was bumped. Family and past histories were irrelevant. Serologic tests for syphilis were negative. The tumor was excised, but the inguinal nodes were not removed. The pathological diagnosis was granular-cell myoblastoma of the right thigh. The patient entered Denver General Hospital in August, 1952. The inguinal nodes had greatly enlarged since her discharge from the Corwin Hospital in July, 1949. They were painful, hard, and fixed to the underlying tissues. A chest x-ray at that time showed two well-defined, rounded densities in the left lung at the level of the second and third anterior and intercostal spaces. These were interpreted as metastases from the inguinal tumor. A biopsy specimen was taken from the largest inguinal mass, and a pathological diagnosis of malignant granular-cell myoblastoma was given. The patient gave birth to a full-term female infant at Colorado General Hospital on June 14, 1954. At that time, the inguinal nodes were fused, forming a hard, fixed, multinodular mass, extending from the upper third of the anterior aspect of the right thigh to the right lower abdominal quadrant, 4 in. (10.16 cm.) above the inguinal ligament. The pulmonary densities had also increased in size. Biopsy tissue was taken from the lowermost portion of the inguinal mass. The biopsy specimen measured 25×16×10mm. The cut surface was firm and gray-white.

On histologic examination, the tumor consisted of large, polyhedral cells with markedly granular acidophilic cytoplasm. The cell borders were fairly distinct except in places where the cells seemed to fuse and appeared as very large forms with two or more nuclei. The latter were usually central, single, finely chromatinized, round to oval, and possessed fairly distinct nucleoli. Isolated skeletal muscle fibers mingled with the granular cells in the lower margin of the tumor. Some areas exhibited mild to moderate disorientation of cells with comparatively large, slightly darker, round, oval, or oblong nuclei. A few cells in these fields contained two or more nuclei. Occasional mitotic figures were present. Reticulum stain revealed interlocking slender connective tissue fibers incompletely surrounding groups of tumor cells. Gomori's trichrome tinged the granular cytoplasm light purple-green. No striations were found. Fat and mucicarmine stains were negative.

Course.—The patient was last seen on Aug. 15, 1954. She complained of weakness, cough, and considerable loss of weight. She refused further roentgen therapy.

Submitted for publication Aug. 29, 1955.

Department of Pathology, General Rose Memorial Hospital.

TABLE 1.—*Clinical Features of Clinically Malignant, Histologically Benign Granular-Cell Myoblastomas*

Yr., Author, Case No.	Age, Sex, Race	Location	Size, Cm.	Signs and Symptoms	Therapy	Subsequent History	Duration of Illness, Mo.
1945, Ravich and others [1]	31, male, white	Urinary bladder	12×11×9	Painless hematuria and urinary frequency	Excision, x-ray	Died 17 mo. after initial operation with recurrence and metastases to lymph nodes, bones, liver, spleen, lung, pericardium, peritoneum, prostate	17
1946, Powell,[2] Case 4	26, female, Negro	Subcutaneous nodules in scalp, eyelid, lip, axilla, thigh, and vulva	Up to 4	Multiple skin nodules	Excision	Metastases to ovaries and retroperitoneum; alive 11 mo. after onset	11
1951, Schwidde and others [3]	21, female, white	Right thigh	10×5×4	Lump in right thigh	Excision, x-ray	Cerebral metastases after 6 mo.; metastases to neck of right humerus after 37 mo.	37
1953, Crawford and De Bakey,[4] Case 1	50, female, Negro	Breast	4	Painful lump in left breast	Supportive	Metastases to left lung, liver, and celiac axis after 8 mo.; died in 12th mo.	12
1953, Crawford and De Bakey,[4] Case 2	31, female, Negro	Subcutaneous, over trunk and extremities	Unknown	Symptomless lumps over skin of trunk and extremities	Excision, x-ray	Metastases to uterus and ovaries after 14 yr.; to tongue, lower lip, vulva, esophagus, and neck after 20 yr.	240
1955, Gamboa	30, female, Negro	Right thigh	15	Painful tumor in right thigh	Excision, x-ray	Metastases to inguinal lymph nodes and to left lung after 3 yr.	60

TABLE 2.—*Clinical Features of Clinically and Histologically Malignant Granular-Cell Myoblastomas*

Yr., Author, Case No.	Age, Sex, Race	Location	Size, Cm.	Signs and Symptoms	Therapy	Subsequent History	Duration of Illness, Mo.
1948, Dunnington,[5] Case 2	40, male, white	Eyelid	2×3	Swelling and redness of eyelid	Excision	Generalized metastases after 2 yr.	24
1949, Ceelen [6]	45, female, German	Left arm	10	Multiple tumors of left arm	Excision	Metastases to axillary lymph nodes, breast, and lungs after 1 yr.	54
1952, Ross and others,[7] Case 1	60, male, Polish	Lumbosacral area	2	Swelling in right lumbosacral area	Excision	Recurrence and metastases to inguinal lymph nodes and lungs after 3 yr.	36
1952, Ross and others,[7] Case 2	58, female, Italian	Skin of left ankle	2	Painful swelling over left ankle	Excision, x-ray	Recurrence and metastases to inguinal lymph nodes after 5 yr.; died after 8 yr.	96
1952, Ross and others,[7] Case 3	33, female, unknown	Subcutaneous tumor of left thigh	1.5×2	Asymptomatic lump over left thigh	Excision	Recurred 5 mo. after initial excision; no recurrence or metastases after 2d operation	8

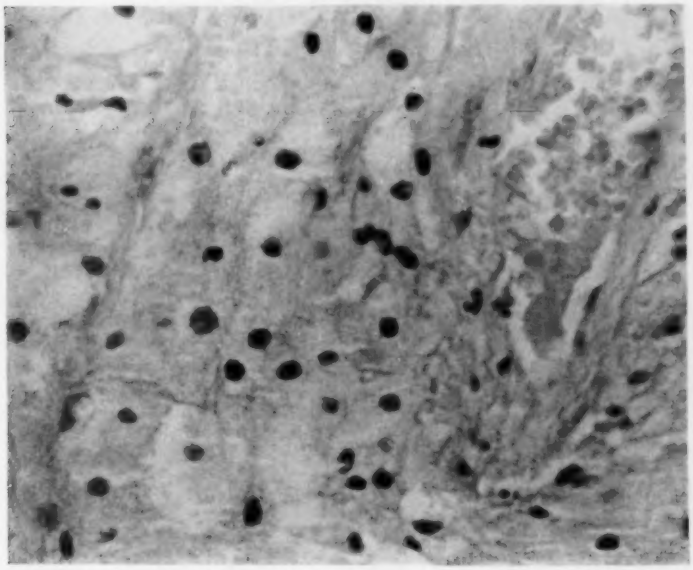

Fig. 1.—Representative area of the tumor. This uniform appearance of the granular cells is very similar to that of benign granular-cell myoblastomas. Hematoxylin and eosin; × 250.

COMMENT

The cases reported as malignant granular-cell myoblastoma by von Meyenburg,[8] Cioni,[9] and Loi[10]; Cases 1, 3, 5, 6, 7, and 9 of Howe and Warren[11]; Cases 2 and 3 of Khanolkar,[12] and Cases 8, 9, and 10 of Murphy, Dockerty, and Broders[13] were rejected because the photomicrographs and descriptions strongly suggested that they were actually rhabdomyosarcomas. Nowhere in these papers did the authors describe the presence of the typical granular cells which are highly characteristic of granular-cell myoblastoma.

The cases reported as malignant granular-cell myoblastoma by Horn and Stout,[14] Hartz,[15] Ackerman and Phelps[16] and Cases 1, 4, 5, and 6 of Khanolkar[12] differed from

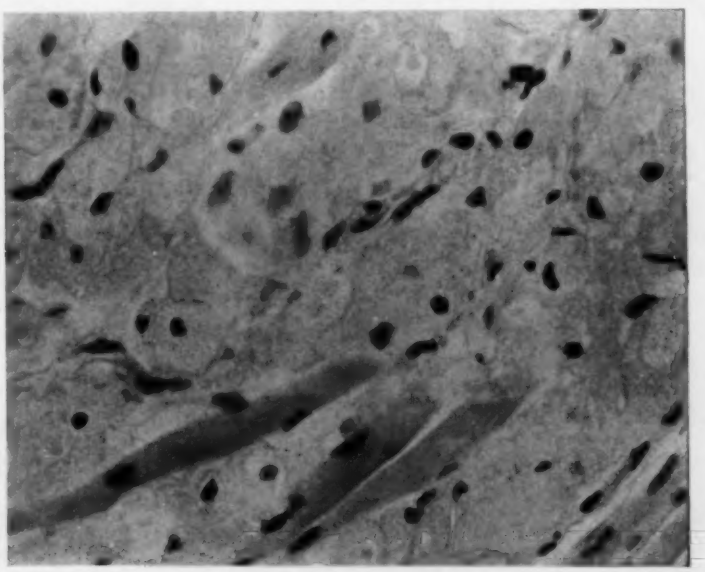

Fig. 2.—Lower margin of the tumor shows a few skeletal muscle fibers mingled with typical granular cells. Hematoxylin and eosin; × 250.

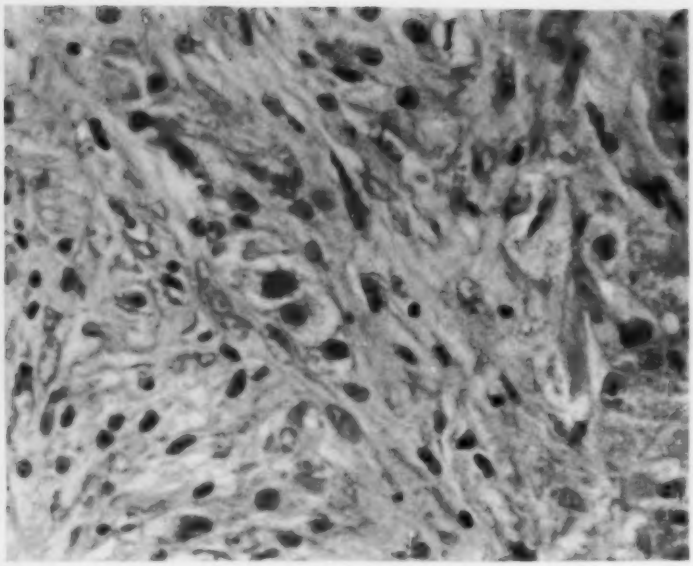

Fig. 3.—One of the few areas showing a mitotic figure and mildly disoriented cells with relatively large and darker nuclei. The neoplastic cells are crowded by fibrous connective tissue. Hematoxylin and eosin; × 250.

true granular-cell myoblastoma in morphology and clinical behavior. From the photomicrographs and descriptions, it was apparent that those tumors possessed an organoid or alveolar-like pattern and that the cytoplasm was vacuolated rather than solid and typically granular. The true granular-cell myoblastomas did not show an organoid pattern, and the cytoplasm was not vacuolated. Cases 1 and 2 of Horn and Stout and Cases 4, 5, and 6 of Khanolkar were probably tumors of the glomus jugulare, while the tumors reported by Hartz, by Ackerman and Phelps, and in Case 1 of Khanolkar were markedly similar to those described by Smetana and Scott [17] as malignant nonchromaffin paragangliomas of the thigh, and by Christopherson, Foote, and Stewart [18] as

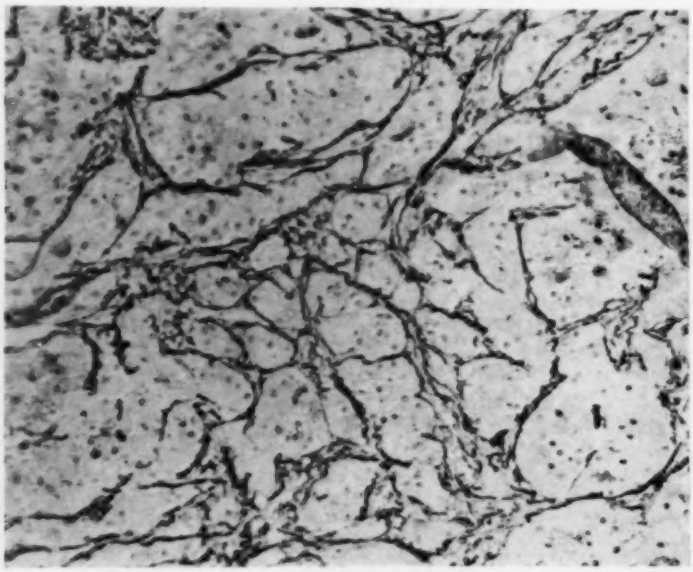

Fig. 4.—Reticulum stain shows interlocking connective tissue fibers incompletely surrounding groups of neoplastic cells. × 60.

alveolar soft-part sarcomas. The clinical behavior was also different because the true malignant granular-cell myoblastomas were fatal within five years by the production of widespread metastases (Tables 1 and 2), while the malignant tumors of the glomus jugulare and other nonchromaffin paraganglia produced repeated recurrences and local invasion. Many of the patients were cured by surgery or died as a result of the tumor only after 15 or more years.

Clinical-Morphological Features. — There were two types of true malignant granular-cell myoblastoma. The first form, which is designated here as the "clinically malignant, histologically benign type" (Table 1), showed the following characteristics. The patients' average age was 31 years. Five of the patients were women; only one was a man. Four were Negroes and two were white. The average size of the tumors was 9 cm. in diameter. They were usually nonencapsulated, and the cut surface was solid, firm, and grayish-white. Microscopically, the pattern was fairly uniform and the component cells were generally identical with those of the ordinary benign granular-cell myoblastomas. However, there were some areas wherein the cells showed mild disorientation and the nuclei were relatively larger and slightly darker than those of the benign tumors. Mitoses were found occasionally. The general appearance was misleading because their malignancy was proved by extensive metastases within three years in five of the six cases. Case 2 of Crawford and De Bakey [4] showed metastases to the uterus and ovaries after 14 years.

The second form, which is labeled here as the "clinically and histologically malignant type" (Table 2), showed the following characteristics. The patients' average age was 47 years. Two were men and three were women. The average size of the tumors was 3.6 cm. in diameter. They were not encapsulated, and the cut surface was firm and grayish-white. Microscopically, they exhibited transitions from the typical granular cells which made it possible to identify their true nature, through areas with well-preserved granularity of the cells but with beginning pleomorphism of nuclei to the extreme variants that consisted of pleomorphic giant cells and spindle cells with nongranular cytoplasm and numerous mitotic figures. They were very malignant, and four of the five cases reported showed extensive metastases within five years. The last case of Ross, Miller, and Foote [7] recurred five months after initial excision but did not recur or metastasize after the second operation.

Origin.—This subject is far too lengthy to discuss adequately in a case report. Suffice it to say that among the major theories, namely, the myoblastic theory, the degeneration of skeletal muscle theory, the storage cell theory, and the neural theory, the last seems to gain the most supporters at present. The papers written by Fust and Custer,* Ashburn and Rodger,[21] Pearse,[22] Feyrter,† Thoren,[25] and Bangle ‡ are mainly responsible for this phenomenon. In my own mind, the fact that some investigators § have found the granular cells within nerve sheaths without the presence of similar cells in the immediately surrounding tissue, in several instances, is good proof that at least many of these tumors were of nervous origin. Whether the Schwann cell or the fibroblast is concerned is difficult to resolve.

Prognosis and Therapy.—The prognosis is the same for both the histologically benign and the histologically malignant types. The patients usually die of generalized metastases within five years. The tumors seem to prefer the lymphatic mode of spread, but metastases by the blood stream are also common. In order of frequency, the secondary growths establish themselves in the regional lymph nodes (64%), lungs (45%), liver (28%), and bones (28%).

These tumors do not respond to irradiation. They are best treated by wide excision

* References 19 and 20.

† References 23 and 24.

‡ References 26 and 27.

§ References 12, 20, 21, 26, and 27.

of the primary tumor and dissection of the regional lymph nodes when early metastasis is suspected.

SUMMARY

The 11th case of true malignant granular-cell myoblastoma is reported.

The tumors may simulate the histologic characteristics of the ordinary, benign granular-cell myoblastomas or may be frankly malignant in microscopic appearance.

The tumors are almost uniformly fatal. They do not respond to irradiation, and it is suggested that they be treated by wide excision of the primary lesion and dissection of the regional lymph nodes when early metastasis is suspected.

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Liver Histochemistry (Mucopolysaccharides) and Plasma alpha$_3$-Globulin

Studies in Diffuse Massive (Fulminant Viral Hepatitis) and Zonal Liver Necrosis (Toxic Hepatitis)

BERNARDO LEO WAJCHENBERG, M.D.
and
GUNTHER HOXTER, M.S., São Paulo, Brazil

In 1 out of 10 cases of fulminant viral hepatitis we have observed a new peak in the electrophoretic pattern (free electrophoresis) of the plasma proteins between the two α-globulins.[1] This fraction was called α_3-globulin.[1]

Incidentally the same peak was observed by Mehl and co-workers[2] in one case of pneumonia, which fraction they called α-x-globulin.

The qualitative analysis of this fraction,[1] in our cases, proved it to be a mucoprotein, that is, a protein-carbohydrate compound with relatively high protein content.

In Mehl's case there was an increase in the mucoprotein value in the blood, not only in the protein (tyrosine) content but also in the carbohydrate moiety.

Because of these facts we have determined the polysaccharide levels of serum in some of our cases of fulminant hepatitis.[3] A normal polysaccharide content was observed, including the mucoproteins, and the results were explained on the basis of the equilibrium of two groups of factors acting upon the polysaccharide level in the serum:

(1) factors which tend to release excessive amounts of polysaccharides into the blood stream: inflammation, stress, and liver necrosis;
(2) factors which tend to decrease their level: impairment of the liver function.

The protein component of the mucoprotein could be normal in "quantity" because the tissue necrosis might compensate for the reduction of the liver function. But some of the protein would then have an altered "composition" because of its liberation through liver necrosis; because of this qualitative difference, it would run in the electrophoretic pattern as a new peak.[3]

Therefore the next step would be the search for a protein-carbohydrate complex in the livers of patients with fulminant hepatitis and a comparison with other types of liver necrosis.

In relation to tissue mucoproteins, Gersh and Catchpole[4] first made the hypothesis that an increase in water-soluble mucopolysaccharides in the connective tissue of one region could produce an increased blood level of water-soluble polysaccharides (muco-proteins) through depolymerization of the ground substance.

Catchpole[5] later, using Winzler's method for mucoproteins,[6] which we used in our previous work, demonstrated that fact in cancer induced in rats.

Nevertheless, this does not prove that we have the same substance in the blood and tissue.

The reason, perhaps, that there is no evidence for the presence of these so-called serum mucoproteins in the tissues is that no one has yet tried to extract and characterize them chemically.

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First Medical Clinic, University of São Paulo Medical School.

American College of Physicians Fellow presently assigned to the Metabolic Research Unit, Department of Internal Medicine, University of Michigan, Ann Arbor, Mich. (Dr. Wajchenberg).

J. Gross, in a discussion about the polysaccharides of mesodermal tissues [7] based on observations related to the permeability of the mucoproteins through the blood vessel wall, stated that the so-called serum mucoproteins could be found in tissues despite no experimental evidence.

The mucoproteins may be identified by tests which reveal the carbohydrate moiety of the molecule. Thus Schiff's reagent for aldehydes gives an intense purple color.[8]

The results obtained by the periodic acid-Schiff reaction will be reported at this time.

MATERIAL

Our material consists of five cases (Cases 1-5) of fulminant viral hepatitis, two cases (Cases 6 and 7) of toxic liver necrosis due to phosphorus poisoning, and one case of benign viral hepatitis.

The diagnosis of fulminant viral hepatitis was made according to Lucke and Mallory's histologic description,[9] which all of these cases presented.

It should be mentioned that in Case 4 the patient did not have a massive necrosis, but had, rather, a massive cellular degeneration with the cells, near the central vein, presenting vacuolation with apparently normal nuclei.

The two cases with toxic hepatitis presented the well-known perilobular necrosis.

For comparison we studied two normal livers and one case of uncomplicated viral hepatitis.

All the livers, except in Case 1, were obtained within 30 minutes after death, the two normal and the benign viral hepatitis tissue fragments being obtained by surgical biopsy. In Case 1 needle biopsy was done.

HISTOCHEMICAL METHODS

The liver samples were fixed in cold alcohol-formal-acetic acid and stained with Schiff's leucofuchsin reagent of the oxidation with periodic acid (McManus-Hotchkiss*). Since this reagent also stains glycogen, the alcohol-insoluble carbohydrate complexes (polysaccharides) were differentiated from the former by the saliva test, which dissolves glycogen but leaves the other carbohydrate unaffected.

The tissue sections were counterstained with malachite green.

Acetylation was performed according to the method of McManus and Cason.[12]

Besides these techniques, the slides were also treated in the following manner: (1) hematoxylin-eosin; (2) silver stain for reticulin fibers, and (3) fixation in formalin-calcium and staining with Sudan-black B for lipids.

For histologic demonstration of ceroid the following staining methods were used in some of the sections: (1) Ziehl-Neelsen method [13]; (2) Sudan III and Sudan-black B.[13]

Unstained sections of Case 5, one of the fulminant viral hepatitis cases, were examined by fluorescent microscopy.

The excitation was centered around 405mμ.

The primary filter was Corning C. S. No. 5-58 (Glass No. 5113), which has the advantage of transmitting no visible light at a wavelength greater than that of about 500mμ. The secondary filter, used to remove short-wavelength light at about 400mμ, while transmitting longer wavelengths, was a gelatin filter Wratten G. Interference filter used was Baird 4050 A.

RESULTS

1. Electrophoretic Pattern of the Plasma Proteins

The electrophoretic patterns of plasma proteins were carried out in Tiselius apparatus at pH 8.6 in barbital sodium and in a constant electric field of 8 volts per centimeter, during 6000 seconds. The optic Philpot-Svenson system was used. The areas corresponding to each fraction were calculated geometrically, and the sum of the areas was always compared with the total proteins obtained through the amount of protein nitrogen (micro-Kjeldahl method). The plasma for electrophoresis was drawn from 14 hours to several minutes before death.

The electrophoresis of the plasma proteins of our cases of liver necrosis is shown in Table 1, where the figures represent a single determination per patient.

As we can see from Table 1, the fulminant viral hepatitis cases had generally low albumin levels, the α_1-globulin being normal. We did not notice an increase in α_2-globulin, but rather a decrease in two of our cases (Cases 3 and 4), despite the knowledge that the α_2-globulins are increased in diseases associated with tissue destruction.[14]

The α_3-globulin appeared in all our fulminant cases except Patient 2. Low β-globulin and variable values of fibrinogen and γ-globulin were observed.

* References 10 and 11.

TABLE 1.—*Electrophoretic Pattern of the Plasma Proteins in Cases of Fulminant Viral and Toxic Hepatitis*

	Diagnosis	Albumin	α_1	α_2	α_3	β	Fibrinogen	γ	Total
Normal* controls, mean	.....	4.70	0.30	....	0.55	0.90	0.40	0.90	7.75
Standard deviation	.....	±0.38	±0.10	....	±0.12	±0.12	±0.15	±0.24	±0.69
Case 1	Fulminant hepatitis	3.32	0.30	0.19	0.40	0.58	0.23	0.70	5.72
Case 2	Fulminant hepatitis	4.70	0.29	....	0.48	0.54	0.53	1.50	8.04
Case 3†	Fulminant hepatitis	1.98	0.20	0.05	0.21	0.30	....	3.91	6.65
Case 4	Fulminant hepatitis	1.80	0.21	0.21	0.18	0.26	0.41	0.02	3.89
Case 5	Fulminant hepatitis	3.46	0.25	0.40	0.54	0.52	0.40	1.38	7.05
Case 6	Toxic hepatitis	3.04	0.08	....	0.31	0.94	0.16	0.25	4.78
Case 7	Toxic hepatitis	2.75	0.09	....	0.38	1.00	0.08	0.09	5.59

* Figures based on 20 healthy adults, 10 males and 10 females.
† In Case 3 the electrophoresis was done in the serum.

The two cases of acute phosphorus poisoning presented a low albumin level and very low values of α_1-globulins, fibrinogen, and γ; the latter could be explained by the renal lesions. A normal value of β-globulin was observed in one case, and a high value was observed in the other. In neither did we have the α_3-globulin.

2. OBSERVATIONS ON LIVER GLYCOGEN (TABLE 2)

The normal liver contains stainable glycogen that appears as globular droplets which are clearly visible under large magnifications. The cells were arranged regularly and packed with glycogen.

In the case of benign viral hepatitis (Case 8), the individual cells were almost as full of glycogen as those in the normal tissue, but they were forced apart by prominent interstitial tissue and, instead of being arranged regularly, the cells here and there were scattered.

The two cases of toxic hepatitis revealed that glycogen had been completely lost from both the necrotic and the surviving cells.

In three of our fulminant cases (Cases 1, 2, and 5) the parenchymal cell ghosts were practically empty of glycogen.

In Case 1, without liver glycogen, the tissue fragments were obtained by needle biopsy, as previously mentioned, while the patient was still alive in order to have an accurate picture of the glycogen because of the known rapidity of postmortem glycogenolysis; but the patient was in agonal state, and it is also known that even brief agonal states cause drastic changes in the liver glycogen of animals.[15]

A remarkable fact was observed in Cases 3 and 4, in which PAS-positive material, as very fine particles, was observed scattered through the sections, out of the cells, surviving or necrotic (Fig. 1), and this material disappeared completely after saliva treatment. We do not know any substances other than glycogen that show the staining properties just mentioned.

TABLE 2.—*Hepatic Histochemistry (Glycogen and PAS+ Material Nonglycogen) in Cases of Fulminant and Benign Viral and Toxic Hepatitis*

Diagnosis	Case No.	Glycogen	PAS+ Material
Normal		++++ (Intracellular)	.....
Fulminant hepatitis	1	.....	+++ (Intracellular)
	2	.....	+++ (Intracellular)
	3	+ (Extracellular)	+++ (Intracellular)
	4	++ (Extracellular)	++ (Intracellular)
	5	.....	++ (Intracellular)
Toxic fatal hepatitis	6	.....	+ (Intracellular, periportal)
	7	.....	+ (Intracellular, periportal)
Benign viral hepatitis	8	+++ (Intracellular)	.....

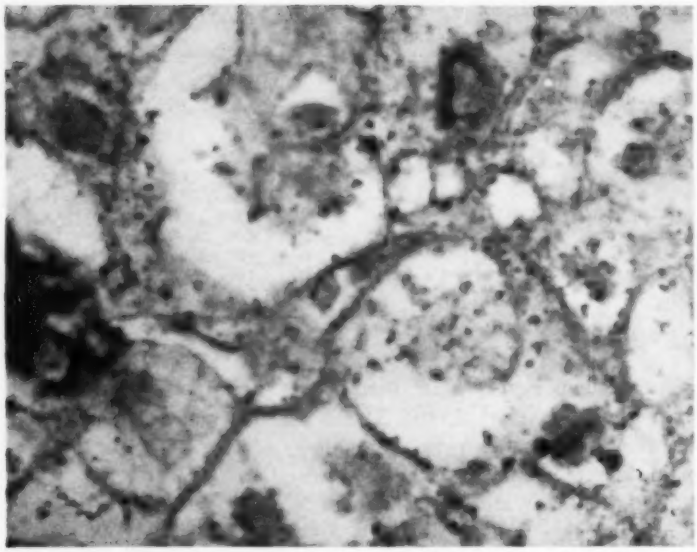

Fig. 1 (Case 4, fulminant hepatitis). — Liver section treated with the PAS method and counterstained with malachite green. PAS-positive material, as very fine particles, scattered through the section, principally out of the necrotic cells; reduced slightly from mag. × 517.

3. Observations on Other PAS-Positive Materials, Following Digestion of Glycogen, in the Liver Sections

The reticulin fibers gave a faint positive reaction, as has been observed by Lillie,[16] due to the presence of a carbohydrate fraction. In all fulminant cases the reticular network was conserved but fragmented, without collapse.

In the two cases of phosphorus poisoning the reticulin was fragmented in the necrotic areas.

In the fulminant hepatitis cases and in the necrotic cells about the portal areas in the toxic cases, PAS+ granules were observed in the degenerating or necrotic liver cells (Fig. 2). All this material was intracellular and that which was extracellular disappeared after saliva treatment, as has been shown before.

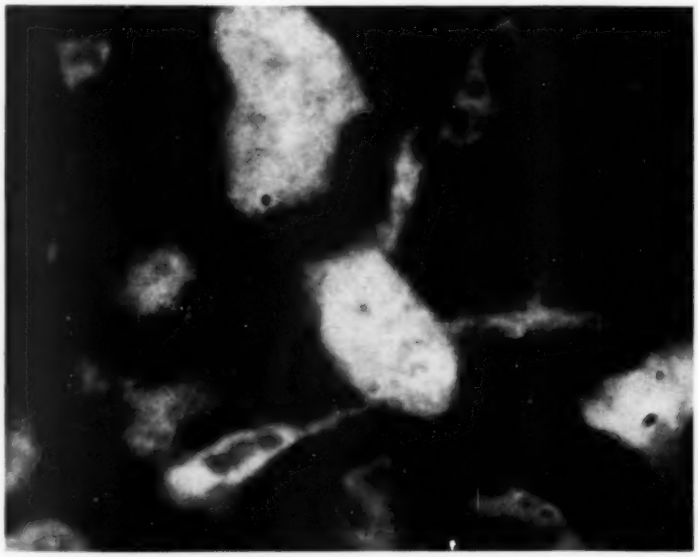

Fig. 2 (Case 5, fulminant hepatitis).—Unstained liver section in fluorescence microscopy, showing white granules, without clearcut limits, side by side, with darker granules (?lipogenic pigments); reduced slightly from mag. × 500.

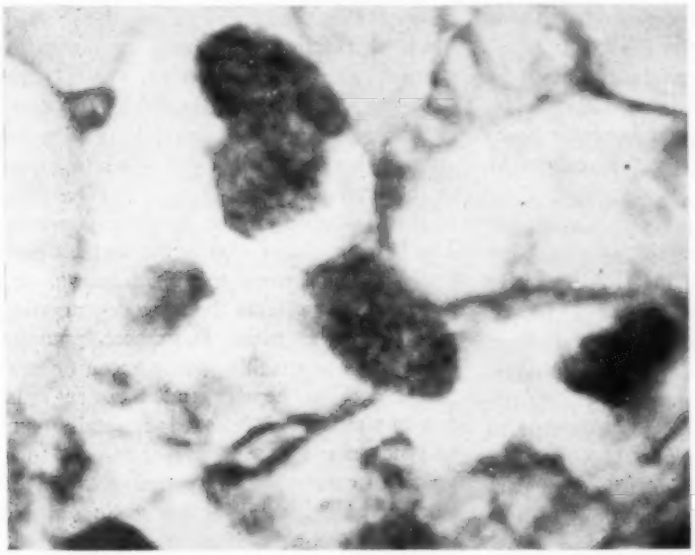

Fig. 3 (Case 5, fulminant hepatitis).—Same liver section as shown in Fig. 2, treated with saliva and stained by the PAS method, counterstained with malachite green. PAS+ granules in two degenerating cells, with a clear central space, together with brown granules (?lipogenic pigments); reduced slightly from mag. × 500.

In Cases 1 and 3 this material was scattered through the section, but in Cases 2, 4, and 5 the distribution was more focal than diffuse.

These granules showed a rim which stained bright red. The greater portion, which was within the rim, stained faintly.

Opie [17] gave the name of cytochondria to the vesicular bodies presented principally in the cytoplasma of liver cells undergoing pathological changes and with definable rim and clear central space (Fig. 3). The granules were observed to be water-soluble.

These granules were quite regularly accompanied by a brown pigment (Fig. 3) similar to that described by Lucké and Mallory [9] (lipofuchsin) in fulminant cases of viral hepatitis, and also described by Lucké in livers from patients cured of the disease.[18]

It should be noted that several brown pigments, which stain by Sudan III, as lipofuchsin, have been described in the liver and other tissues.

Gomori [19] terms all these granules as the "lipogenic pigments," because of their relation to lipid metabolism.

In one of the fulminant cases (Case 5), the PAS+ granules were studied to prove whether they were or were not ceroid, one of the lipogenic pigments; they were stained by Sudan III and Sudan-black B but not by the Ziehl-Neelsen method, therefore not acid-fast.

Acetylation blocked the PAS reaction of the granules, but treatment with 0.1 N KOH for 15 minutes after acetylation followed by the PAS reaction restored the color of the reactive material.

Finally, in the fluorescent microscope, the granules appeared as white-yellow, without clear-cut limits (Fig. 2). The fluorescence was stable. No red porphyrin fluorescence was seen in the liver.

COMMENT

The comments will be divided into two parts: (1) nature of the PAS+ granules observed in the hepatic cells and (2) correlations of the histochemical findings with the a_2- and a_3-globulins.

1. Nature of the PAS+ Granules Observed in the Hepatic Cells.

The exact nature of this material in the degenerating liver cells is not known. Prior treatment with saliva did not affect the stainability.

Insofar as we know, this is the way to detect alcohol-insoluble carbohydrate complexes and other substances containing the

1,2-glycol grouping (or the equivalent amino or alkylamino derivatives).

The glycolic groups can be produced by the oxidation of unsaturated fatty acids; therefore the possibility that insoluble lipids may be involved in the reaction cannot be excluded.†

According to McManus and Cason, the acetylation technique should establish the carbohydrate nature of the PAS-positive material.

Pearse,[21] however, has expressed some doubt on the usefulness of this technique.

Anyway we should consider the possibility that the pigment might be related to ceroid, which also gives a PAS+ stain, and might similarly be formed through partial oxidation and polymerization of unsaturated fatty acids,[22] the mechanism suggested for the formation of ceroid. The evidence that ceroid is a derivative of fatty acids seems conclusive, but that it is a result of necrosis is debatable. It is suggested that both necrosis and ceroid deposition are the result of the same primary process, that is, altered metabolism of the unsaturated fatty acids.[23]

Nevertheless, it should be pointed out that the granules in our cases differ somewhat from ceroid in the respect that they are not acid-fast.

It could be suggested that this PAS+ material might perhaps be related to the other lipogenic pigments, as lipofuchsin, lipochrome, etc. As it happens with the ceroid, all these pigments have alcohol-acid fastness of fuchsin-staining and being lipidic constituents are water-insoluble, whereas the PAS granules are, as was previously mentioned, water-soluble.

From all these data we believe that the PAS+ granules, in our present series, are probably of a different substance than the lipogenic pigments; therefore these observations confirm the results with the acetylation technique.

It should be noted that the Sudan-black B studies in three of our fulminant cases showed the predominance of the Sudan-black B reacting substances in an extracellular situation, therefore, with quite different distribution of the PAS+ material.

In considering the ultraviolet fluorescent studies, we found the yellow-white aspect of the granules (Fig. 2) to be quite similar to that described for the lipogenic pigments. The latter could be seen in the sections generally as dull-brown to golden-brown pigments, side by side, in the same degenerating cells with the white fluorescent granules (Fig. 2). The quickly fading green fluorescence, both in hepatic and Kupffer cells, due to vitamin A,[24] which is normally seen, was not noted in our preparations. However this cannot be interpreted because the mounting media used for our sections would dissolve vitamin A, if present. Despite the same histochemical properties, the PAS+ granules were also different from the pigment described by Dubin and Johnson,[25] both in the appearance on hematoxylin-eosin sections and in the predominant centrolobular distribution of the latter.

Another differential feature is the dull-brown fluorescence emitted by this pigment under ultraviolet light.

It should also be noted that we can have emission of different colors in the fluorescent microscope according to the filters used. Unhappily, Dubin and Johnson's work and that of many others do not mention the type of filters used, and because of this our data on ultraviolet fluorescence cannot be compared with much of the published data.

In conclusion, it is more probable that the PAS+ material is a polysaccharide complex, although we cannot definitively rule out the possibility that it may be related to the lipochromes.

If it is a polysaccharide-containing material, considering the strong stainability with PAS, the carbohydrate moiety would be a neutral or basic mucopolysaccharide.[26]

Braden,[26] working with isolated mucopolysaccharides, showed that all acid mucopolysaccharides from connective tissue

† References 20 and 21.

and heparin trisulfate did not stain or stained very slightly with the PAS technique.

In contrast, each of the three neutral mucopolysaccharides or mucoproteins from hog gastric mucin which have been tested by the PAS test were found to be strongly positive.

However, many known sites of the acid mucopolysaccharides, as the ground substance of connective tissue, stain strongly by the PAS. Braden [26] answers that it is suggested that hyaluronic acid, the principal constituent of the ground substance, in its native state is often associated with a nonmetachromatic PAS-reactive substance, possibly a neutral mucopolysaccharide. This would explain the observations of Bunting [27] and Braden [26] that the PAS staining is unaffected by incubation of the sections in hyaluronidase, though the metachromasia of these tissues is readily removed thereby.

Later we will continue this discussion.

2. Correlation of the Histochemical Finding with the α_2- and α_3-Globulins

Increase in the α-globulins of human sera has long been associated with "tissue destruction irrespective of its cause," [14] the increase being essentially due to the glycoproteins.[28]

Thus in myocardial infarction the characteristic electrophoretic aspect is the persistent increase in the α-globulins and also in the polysaccharides of the serum.[29]

In experimental myocardial infarction it has been shown that there is a marked reduction in glycogen in the ischemic area, and, in addition, a peculiar degenerative change occurs in ischemic fibers within a few hours following vascular occlusion.[30] It is characterized by a positive PAS reaction within the fiber, which becomes progressively darker with time. This reaction is not due to glycogen, because prior treatment with saliva did not affect the stainability.[30]

Therefore we have had similar findings in the liver of our cases, in the appearance of PAS+ material, not glycogen.

That this is a generalized phenomenon and not specific for the two mentioned organs is shown by the increase in the concentration of α_1- and, principally, α_2-globulins in choline-deficient rats with degeneration of the kidney tissue.[31] The experiments done have shown a direct relationship between the α_2-globulin area changes in the electrophoretic pattern and the kidney lesions.

However, the large number of unrelated conditions which result in increases in protein-carbohydrates of serum prompted Boas and Peterman [32] to propose that the elevated serum glycoprotein or polysaccharide levels may also represent a response to nonspecific stress.

This separation of tissue anabolic and catabolic factors in several cases presents some difficulties.

Thus, in myocardial infarction and kidney necrosis we have clearly the two factors: tissue destruction and protein synthesis related to response to stress, both reflected in the high protein-polysaccharide levels in the blood.

We should expect in these two conditions because of the tissue necrosis the appearance of new peak(s) in the electrophoretic pattern of the plasma proteins, if the tissue proteins were released by the degenerative areas.

The following observation was really noticed: Fischer and Garrity,[31] in 90% of the choline-deficient animals studied, observed that the α_2-fraction was composed of two peaks. The double peak was not observed in the normal or supplemented groups (with choline). Also, Donzelot and co-workers [29] observed in certain cases of myocardial infarction a globulin intermediary between α_2 and β_1, to which they gave the name "α_3-globulin."

In cases of fulminant hepatitis with diffusive massive liver necrosis it was shown in a previous paper [3] that the polysaccharide-bound-protein levels in the serum were normal. This was explained by a decreased protein synthesis due to the liver destruction, and these necrotic phenomena would explain the appearance of a new

globulin, α_3-globulin, which proved, as we might expect, to be a mucoprotein.

There is not enough data in the literature to know whether the PAS+ material in the necrotic areas other than those of the liver have also a granular shape.

At this point two important questions arise: First, what is the significance of the PAS+ granules and, second, is there direct relation between these granules and the α_3- or α_2-globulins and could they result from other parts of the liver than the parenchymal cells which are known to contain polysaccharides, such as the connective tissue?

In connection with the first question it should be mentioned that these granules, as stated above, could correspond to the cytochondria,[17] described by Opie some years ago. According to this author, pathological changes in the liver cells, not only the mitochondria but all of the cytoplasmatic granules (cytochondria), lose their distinctive histochemical reactions, becoming apparently indistinguishable one from the other. "These changes that occur in cytochondria give insight into the pathogenesis of a variety of pathological lesions including the appearance of granules which are intensely reactive to the PAS stain." [33]

It is stated that regenerating renal tubular cells do not develop these granules because they lack mitochondria.[34]

Much evidence has been brought forward that pentosenucleic acid (ribonucleic acid) is related to the cytoplasmatic protein formation,‡ and the so-called basophilic bodies in the cytoplasma have been shown to contain pentosenucleic acid, the phosphoric acid radical being responsible for the basophilia.

The normal liver cells contained numerous basophilic bodies in their cytoplasm. The epithelial cells of the bile ducts contained little or no pentosenucleic acids. The cytoplasm of the Kupffer cells was practically free of basophilic bodies.[37]

‡ References 35 and 36.

Opie [17] assumed a close relation between the cytoplasmic basophilic material and mitochondria.

In ultracentrifugation studies this material is mainly found in the granular (mitochondrial, microsomal, and ultramicrosomal) fraction and little in the supernatant fluid.[38]

In seven instances of the fulminant form of infectious hepatitis,[37] the basophilic bodies were notably decreased or absent from cytoplasm of the hepatic cells in contrast to the mesenchymal elements, which were usually rich in basophilic bodies. Also, in cases of toxic hepatitis [37] these bodies were absent or decreased in the necrotic zone.

Therefore, we could follow the Opie [17] assumption to explain the appearance of the PAS+ granules, with the chemical changes in the mitochondria due to the disappearance or decrease of the pentosenucleic acid and consequent change in the staining behavior. This change could be due to the accumulation of fat within cytochondria when parenchymatous cells undergo degeneration or necrosis,[17] or due to the appearance of polysaccharides which were associated with the nucleic acids; and with the disappearance of the latter the former would become easily demonstrated, following the same reasoning done by Braden,[26] as previously mentioned.

The latter observation is only a possibility and without proof with data at hand.

In relation to the second question, since we do not know for certain that those granules in the liver cells are polysaccharide-containing, we cannot relate them to the α_3-globulin, a carbohydrate-bound protein, as was shown. Besides, in one case of fulminant viral hepatitis (Case 2) in which these granules were demonstrated in the tissue sections, we could not show the new peak in the electrophoretic pattern.

The other fact, already mentioned, is that these granules should probably be neutral or basic mucopolysaccharides, according to Braden.[26]

As shown by Mehl and co-workers,[2] all the mucoproteins, including one case with the α_3-globulin, the α_3-mucoprotein, should be

an acid mucopolysaccharide. Further evidence is suggested by its position between the α_1- and α_2-globulins, fractions with low isoelectric points.

It is not known, however, if Braden's [26] suggestions are correct. If the granules contain polysaccharides, they could be acid and related to the new electrophoretic peak. This would not allow us to adequately interpret Case 2, mentioned above.

In connection with the last part of the second question, the best interpretation was given by Gersh and Catchpole,[4] which emphasized the concept that the serum glycoproteins might arise as a result of the depolymerization of the ground substance of the connective tissue with release of the solubilized components into circulation.

In accordance with this concept we should expect increase in the mucoproteins in the serum of our cases of fulminating hepatitis. This does not occur, as shown in a previous paper.[3]

Another possibility could be that glycogen appears in the blood. This is suggested by its observation in extracellular position in two of our cases. However, the glycogen would not give the mucoprotein reaction.

The best explanation is still that the PAS+ granules appearing in the blood are the new α_3-globulin.

CONCLUSIONS AND SUMMARY

In an attempt to establish the correlation between the hepatic histochemistry with the periodic acid-Schiff (PAS) stain and the α_3-globulin content of the plasma, previously shown to be mucoprotein, in cases of fatal liver necrosis, we studied five cases of fulminant viral hepatitis, two cases of toxic liver necrosis due to phosphorus poisoning, and one case of benign viral hepatitis.

The cytologic distribution of glycogen and other PAS-positive material was described for two normal livers. In the pathological cases, glycogen was observed in intracellular and extracellular situations. PAS-positive material, nonglycogen, was observed as granules, with the characteristic of cytochondria, as described by Opie.[17] By the use of several histochemical techniques and fluorescent microscopy, it was felt that these granules were not lipogenic pigments, more probably being a polysaccharide complex, although we could not rule out completely the former possibility.

It was also suggested that the appearance of PAS+ material, nonglycogen, in degenerating cells was a generalized phenomenon. In support to this concept we reviewed work done by several investigators in heart and kidney necrosis.

These granules would probably result from the mitochondria of the hepatic cells.

No good correlation was observed between these granules and the appearance in the blood of the carbohydrate-bound protein noticed in most cases of fulminant hepatitis (α_3-globulin); however, it should be considered that the tissue sample was always taken after the blood, and we do not know how much the necrotic process did progress during this time.

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Epidemic Gastroenteritis Due to Escherichia Coli O111 B_4

II. Pathologic Anatomy (With Special Reference to the Presence of the Local and Generalized Shwartzman Phenomena)

CAPT. DONALD G. McKAY (MC)
and
LIEUT. GEORGE H. WAHLE Jr. (MC), U. S. Army

During the winter of 1953-1954 an epidemic of infantile diarrhea at Fort Belvoir, Va., took the lives of nine infants. Pathologic examination of these fatalities revealed the fact that in every case histologic evidence of diffuse intravascular clotting could be found. These lesions were identical with those observed in the kidneys of rabbits subjected to the generalized Shwartzman phenomenon.[1] Further consideration of the areas of hemorrhagic necrosis of the mucosa of the small bowel and the colon in these cases led to the idea that these lesions represented the local Shwartzman reaction.[2] The interpretation of these pathologic changes as manifestations of the local and generalized Shwartzman phenomena (*a*) offers an explanation of the mechanism of death in certain infants with infantile diarrhea, (*b*) aids in the understanding of how an enteric infection with Escherichia coli (Serotype O111 B_4) could cause this disease, and (*c*) points to the need for careful studies of the blood-clotting mechanism in the victims of infantile diarrhea.

It is the purpose of this report to describe the pathologic anatomy of the cases in this epidemic, to compare it with the pathologic descriptions from other epidemics, to correlate the pathologic anatomy with the clinical aspects, and to present the evidence that points to the local and generalized Shwartzman phenomena as pathogenetic mechanisms in infantile diarrhea.

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From the Department of Pathology, U. S. Army Hospital, Fort Belvoir, Va.

Present address: Department of Pathology, Harvard Medical School, Boston (Capt. McKay).

PREVIOUS PATHOLOGICAL DESCRIPTIONS

The question as to whether the Fort Belvoir epidemic was similar to the previously reported epidemics may be partially answered by the pathologic findings. A survey of the more recent description of these in epidemic infantile diarrhea reveals, with few exceptions, a notable similarity of findings and strongly suggests that most of these epidemics were the same disease, even though they were called by different names.

In general, the small intestine has shown the most marked alterations. These have consisted of hyperemia and congestion of the mucosa with swelling of Peyer's patches,* patchy hemorrhages with superficial mucosal ulcerations,† blood and bloody mucus in the lumens,[7] dilatation,[8] and swelling of the mesenteric lymph nodes.‡ Similar changes have been observed in the stomach and colon but less frequently.§ Atelectasis, edema, lung abscesses, bronchopneumonia, and hemorrhage have been found in the lungs.‖ Cerebral changes have consisted of edema,¶ small hemorrhages,# perivascular accumulations of

* References 3 and 4.
† References 5 through 12.
‡ References 5 and 9.
§ References 4, 6, 9, and 12.
‖ References 5, 7 through 9, and 11 through 13.
¶ References 4, 6, and 14.
# References 6, 7, and 14.

lymphocytes,* beginning thrombosis of cerebral vessels,[15] leptomeningitis,[14] and degeneration of ganglion cells.[14] Fatty infiltration † and focal necrosis of the liver ‡ have been reported. Hemorrhages have occurred in the spleen,[3] adrenals,§ kidneys,‖ skin,[7] and serosal surfaces.¶ Dehydration,# otitis media,* and peritonitis [10] have been described in a few cases.

Various suggestions as to etiology and pathogenesis of this disease process have appeared. Many authors have considered that these changes could be ascribed to a virus infection.† Israels [12] believed that malnutrition was responsible. Felsen and Wolarsky [8] considered that the gastroenteritis was secondary to a focus of infection elsewhere in the body. Rivers appears to have been the first to recognize that the clinical and pathologic findings in infantile diarrhea could be attributed to certain toxins of the colon bacillus.

REPORT OF CASES

TABLE 1.—*Case 1*

Male Infant, 36 Days Old; Weight at Death 3720 Gm.; Duration of Illness 7 Days; Developed Shock on the 7th Day

Organ	Clinical Findings	Pathological Findings
Kidneys	Terminal albuminuria	Colloid droplet degeneration of proximal tubule near glomerulus; rare eosinophilic cast; uric acid deposits in papillae
Lungs	.....	Edema; focal intra-alveolar hemorrhage; fibrin thrombi
Brain	Terminal coma	Subarachnoid hemorrhage, superior posterior portion left frontal lobe; hyperemia; edema; focal hemorrhages; margination of Nissl substance; fibrin thrombi
Gastrointestinal tract	Paralytic ileus; bloody stools 7th day	Stomach dilated; chronic inflammation with plasma cells, lymphocytes, and polymorphonuclear leucocytes in mucosa Small bowel: distended, chronic inflammation; edema of Auerbach plexi Colon: distended

Cultures: stool, E. coli O111 B_4; blood, no growth
Fibrin thrombi: lungs +; spleen ++; brain +

TABLE 2.—*Case 2*

Female Infant, 13 Days Old; Weight at Death 2075 Gm.; Duration of Illness 7 Days; Dead on Arrival

Organ	Clinical Findings	Pathological Findings
Kidneys	.....	Colloid droplet degeneration of proximal tubule near glomerulus; bile pigment in tubular epithelium; dilation of glomerular capillaries with fibrin thrombi
Lungs	.....	Aspirated vomitus; mucoid debris in bronchi; focal atelectasis; fibrin thrombi
Brain	.....	Subarachnoid hemorrhage; massive intraventricular hemorrhage with rupture of third ventricle; fibrin thrombi
Gastrointestinal tract	.....	Esophagus: mucosa purple Stomach: chronic inflammation of pyloric mucosa Small bowel: dilated; ulceration and hemorrhage of mucosa with blood in lumen; edema of Auerbach plexi Colon: mucosa congested
Liver	.....	Yellow and brown mottling; inspissated bile in canaliculi; focal central necrosis

Fibrin thrombi: lungs ++; spleen +; kidneys +

TABLE 3.—*Case 3*

Male Infant, 30 Days Old; Weight at Death 2500 Gm.; Duration of Illness 17 Days; Developed Shock on 6th and 15th Hospital Days

Organ	Clinical Findings	Pathological Findings
Kidneys	Anuria last 2 days	Hemoglobin casts in tubules; fibrin thrombi; few glomerular abscesses
Lungs	.....	Pulmonary edema and congestion; fibrin thrombi
Brain	Terminal coma	Petechial hemorrhages, edema, congestion, fibrin thrombi; focal acute arteritis and perivascular leucocyte infiltration
Gastrointestinal tract	Paralytic ileus; bloody stools and emesis 15th-17th days	Esophagus and stomach: mucosal congestion Small bowel: edema, congestion of mucosa; hemorrhagic ulcerations of mucosa; chronic inflammation of mucosa Colon: perforation of sigmoid; hemorrhagic ulceration of mucosa
Liver	Icterus	Inspissated bile in canaliculi; slight fatty metamorphosis

Cultures: stool, E. coli O111 B_4; blood, no growth
Fibrin thrombi: lungs +++; adrenals +; kidneys +++; jejunum +; brain +

PATHOLOGIC DESCRIPTION

The body weights at the time of death ranged from 2000 to 5740 gm., and two of the infants were premature. All were emaciated, and one exhibited marked edema of the lower half of the body. Skin petechiae were found in three cases, and cyanosis in two.

* References 6 and 14.
† References 10, 12, and 16.
‡ References 9, 11, and 12.
§ References 3 and 13.
‖ References 7 and 12.
¶ References 7 and 12.
# References 9 and 17.
* References 5 and 12.
† References 3, 6, 7, 10, 11, and 14.

TABLE 4.—*Case 4*

Male Infant, 23 Days Old; Weight at Death 2000 Gm.; Duration of Illness 19 Days; Developed Shock on 4th and 19th Hospital Days

Organ	Clinical Findings	Pathological Findings
Kidneys	Anuria last 2 days	Hemoglobin casts in tubules; vascular congestion; fibrin thrombi
Lungs	.....	Edema, congestion, fibrin thrombi
Brain	Terminal coma	.....
Gastrointestinal tract	Paralytic ileus; bloody stools and emesis 14th and 17th days	Stomach: distention, mucosal petechiae, chronic inflammation Small bowel: distention, hemorrhagic ulceration of mucosa, bloody mucus in lumen, edema of Auerbach plexi, fibrin thrombi in capillaries Colon: same as small bowel
Liver	Icterus	Congestion, inspissated bile in canaliculi

Cultures: stool, E. coli O111 B_4; spinal fluid, A. aerogenes; blood, no growth
Fibrin thrombi: lungs +++; spleen +++; adrenals ++; kidneys ++; ileum +; bladder +

TABLE 5.—*Case 5*

Male Infant, 33 Days Old; Duration of Illness 28 Days; Developed Shock on 20th Hospital Day

Organ	Clinical Findings	Pathological Findings
Kidneys	Anuria last 2 days	Fibrin thrombi; vacuolation of tubular epithelium
Lungs	Hemoptysis 27th and 28th days	Pleural ecchymoses; congestion; acute bronchopneumonia; fibrin thrombi
Brain	Terminal coma	Subarachnoid and subdural hemorrhage, right frontal lobe, cerebellum; perivascular leucocytic infiltrate; fibrin thrombi
Gastrointestinal tract	Bloody stools 28th day; paralytic ileus	Stomach: brown mucus; mucosal hemorrhages Small bowel: chronic inflammation of mucosa; submucosal edema and hemorrhage Colon: bloody mucus; mucosal petechiae with ulceration; submucosal hemorrhage
Liver	.....	Fibrin thrombi

Cultures: stool, A. aerogenes; blood, no growth
Fibrin thrombi: lungs +; liver +++; spleen ++; adrenals ++; kidneys +; brain ++

Peritoneal Cavity.—In general the peritoneal surfaces were smooth and glistening, and in two cases 500 ml. of ascitic fluid was found in the cavity. The small bowel was distended in most cases, and in two instances the serosa was congested.

Pleural Cavity.—Petechial hemorrhages on the visceral pleura were found in two of the infants.

TABLE 6.—*Case 6*

Male Infant, 17 Days Old; Weight at Death 3000 Gm.; Duration of Illness 10 Days; Developed Shock on 9th Hospital Day

Organ	Clinical Findings	Pathological Findings
Kidneys	Anuria last day	Vascular congestion; fibrin thrombi
Lungs	.....	Congestion, atelectasis, fibrin thrombi
Brain	Terminal coma	Petechiae, edema, fibrin thrombi, margination of Nissl substance
Gastrointestinal tract	Paralytic ileus; bloody stools	Small bowel: ulceration, acute and chronic inflammation of mucosa Colon: distention, chronic inflammation of mucosa; fibrin thrombi

Cultures: stool, E. coli O111 B_4; blood, no growth
Fibrin thrombi: lungs +++; adrenals +; kidneys ++++; colon +; brain ++

TABLE 7.—*Case 7*

Female Infant, 180 Days Old; Weight at Death 5740 Gm.; Duration of Illness 46 Days

Organ	Clinical Findings	Pathological Findings
Kidneys	Anuria last 2 days	Acute bacterial glomerulitis; acute pyelonephritis; necrotizing arteritis; monilial abscesses of cortex and medulla; fibrin thrombi
Lungs	Cough	Acute confluent bronchopneumonia
Brain	Terminal coma	Fibrin thrombi in veins and capillaries
Gastrointestinal tract	Bloody stools 45th and 46th days	Esophagus: acute ulceration Small bowel: peritonitis, mucosal ecchymoses, chronic inflammation of mucosa Colon: mucosal hemorrhages with chronic inflammation

Cultures: urine, E. coli (not serotyped); peritoneal fluid, A. aerogenes; blood, Monilia
Fibrin thrombi: adrenals +; kidneys +++; brain +

TABLE 8.—*Case 8*

Male Infant, 47 Days Old; Weight at Death 3330 Gm.; Duration of Illness 40 Days; Developed Shock on 30th Hospital Day

Organ	Clinical Findings	Pathological Findings
Kidneys	Anuria last 2 days	Multiple abscesses of cortex and medulla; tubular necrosis; fibrin thrombi
Lungs	Cough	Ecchymoses, atelectasis, fibrin thrombi
Brain	Convulsions, lethargy, coma	Edema, margination of Nissl substance, fibrin thrombi
Gastrointestinal tract	.....	Stomach: mucosal congestion, ecchymoses and chronic inflammation Small bowel: congestion of mucosa with petechiae and ecchymoses; hemorrhagic ulcerations; chronic inflammation Colon: same as small bowel

Cultures: stool, E. coli O111 B_4; blood, no growth
Fibrin thrombi: lungs +; spleen +; adrenals +; kidneys +++; jejunum ++; ileum ++; brain +++

TABLE 9.—*Case 9*

Female Infant, 27 Days Old; Weight at Death 3350 Gm.; Duration of Illness 13 Days; Developed Shock on 7th and 13th Hospital Days

Organ	Clinical Findings	Pathological Findings
Kidneys	Anuria last 2 days	Congestion, monilial abscess
Lungs	.....	Atelectasis, edema, congestion, focal hemorrhages, fibrin thrombi
Brain	Terminal coma	Acute arteritis, margination of Nisal substance, fibrin thrombi
Gastrointestinal tract	.....	Esophagus: acute ulceration with perforation to left thorax Stomach: blood-tinged mucus, mucosal hemorrhages with ulceration, chronic inflammation Small bowel: distention, bloody mucus, hemorrhagic ulceration of mucosa, chronic inflammation Colon: blood in lumen, hemorrhagic ulceration of mucosa
Liver	.....	Fatty metamorphosis, inspissated bile in canaliculi

Cultures: stool, E. coli (not serotyped); blood, no growth
Fibrin thrombi: lungs +; adrenals +; duodenum +; bladder +; brain +

Pericardium.—Petechial hemorrhages occurred on the epicardium of three cases.

Heart.—In general no changes were observed in the heart. However, one case presented subendocardial hemorrhages, two had focal monilial abscesses in the myocardium, and two others revealed a focal nonspecific myocarditis.

Lungs.—Gross examination revealed many well-demarcated areas of purplish discoloration of the surfaces, and sanguineous fluid exuded from most of the cut surfaces. Microscopically the most outstanding feature was the presence of intravascular fibrin thrombi (Figs. 1 to 3). The majority were in capillaries but a few were found in small arteries, arterioles, and venules. In two instances the larger thrombi were undergoing early organization. The other changes in the majority of cases consisted of atelectasis, vascular congestion, intra-alveolar edema, and hemorrhage. Bronchopneumonia occurred twice.

Liver.—This organ generally was unremarkable, but in three cases inspissated bile was found in the canaliculi, in two cases focal abscesses occurred, and in one a few intrasinusoidal fibrin thrombi were found.

Spleen.—The gross appearance of the spleens was noncontributory. Microscopically the majority contained fibrin thrombi in the sinuses (Fig. 4). One large arterial thrombus was responsible for a splenic infarct (Fig. 5).

Gastrointestinal Tract.—Esophagus: The majority were unremarkable on gross and microscopic examination.

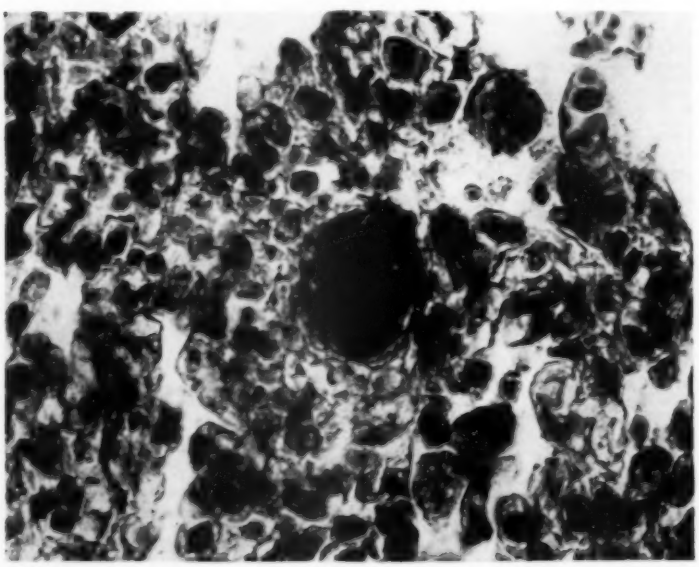

Fig. 1 (Case 6).—Lung. In the center of the field is an intracapillary fibrin thrombus. Phosphotungstic acid hematoxylin; reduced slightly from mag. × 500 (WRAH 14436 C).

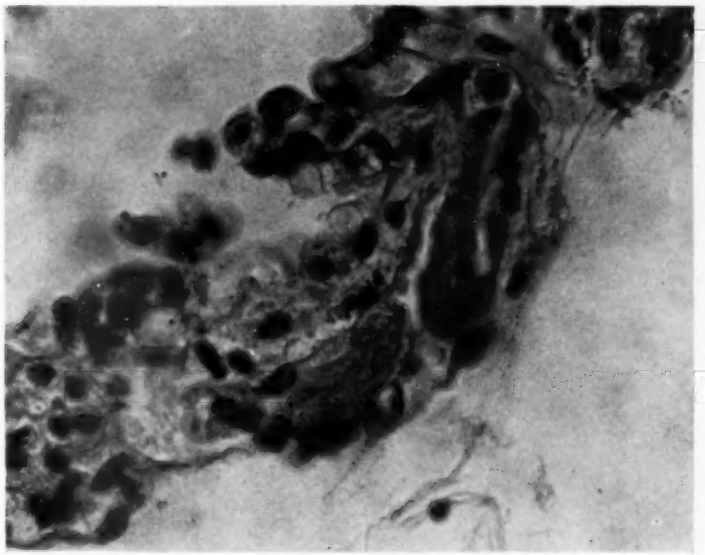

Fig. 2 (Case 3).—Lung. Two intracapillary fibrin thrombi can be seen in this section. Hematoxylin and eosin; reduced slightly from mag. × 500 (WRAH 14435 H).

Stomach: Bloody mucus was present in five cases and was associated with mucosal petechiae in two. Microscopically the mucosa of the fundus was normal, but that of the pylorus was infiltrated with plasma cells, lymphocytes, and macrophages.

Small Intestine: The small bowel of the majority was dilated and in two instances contained bloody mucus. The mucosa was congested and frequently contained petechiae and ecchymoses. Microscopically the mucosa of the entire small bowel in all cases was edematous, and the villous stroma was infiltrated with lymphocytes, plasma cells, macrophages, and eosinophiles (Figs. 6 and 7). Focal hemorrhagic necrosis with loss of the surface epithelium was noted in five cases (Fig. 8). In these regions the mucosal capillaries and submucosal vessels were greatly dilated and in four cases contained fibrin

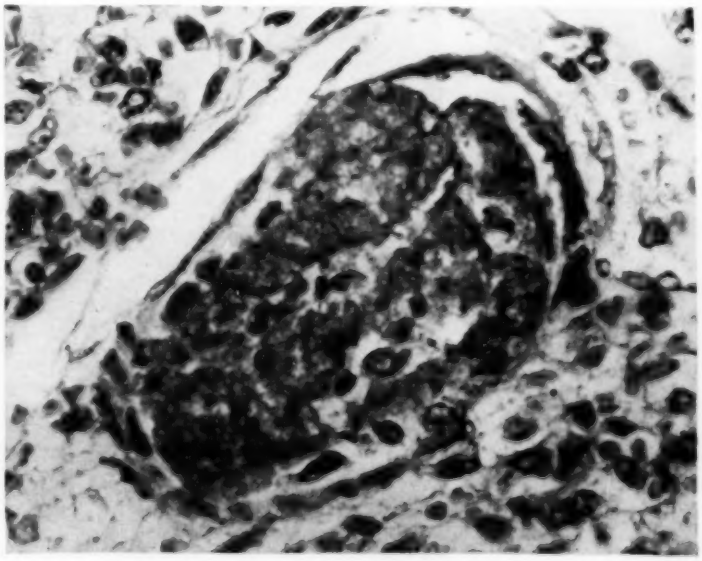

Fig. 3 (Case 4).—Thrombosis of pulmonary venule with numerous masses of agglutinated platelets and scattered strands of fibrin. Hematoxylin and eosin; reduced slightly from mag. × 500 (WRAH 14583 I).

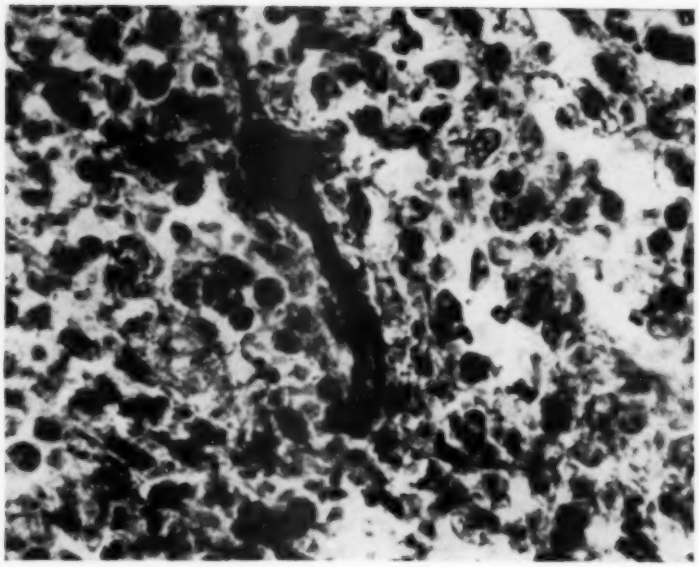

Fig. 4 (Case 4).—Near the center of the field an intrasinusoidal fibrin thrombus of the splenic pulp is demonstrated. Phosphotungstic acid hematoxylin; reduced slightly from mag. × 500 (WRAH 14583 A).

thrombi. There was a marked edema of the muscularis in the region immediately surrounding the plexi of Auerbach.

Colon: The findings in the colon were essentially the same as those in the small bowel and consisted of chronic inflammation of the mucosa, bloody mucus, and hemorrhagic necrosis with ulceration of the mucosa.

Adrenals.—The gross appearance of the adrenals was not contributory. Microscopically the "fetal cortex" exhibited varying degrees of disintegration depending on the age of the infant. These changes were no greater than expected in any infants of comparable ages. Focal hemorrhages and intrasinusoidal fibrin thrombi were found in a few cases (Figs. 9 and 10).

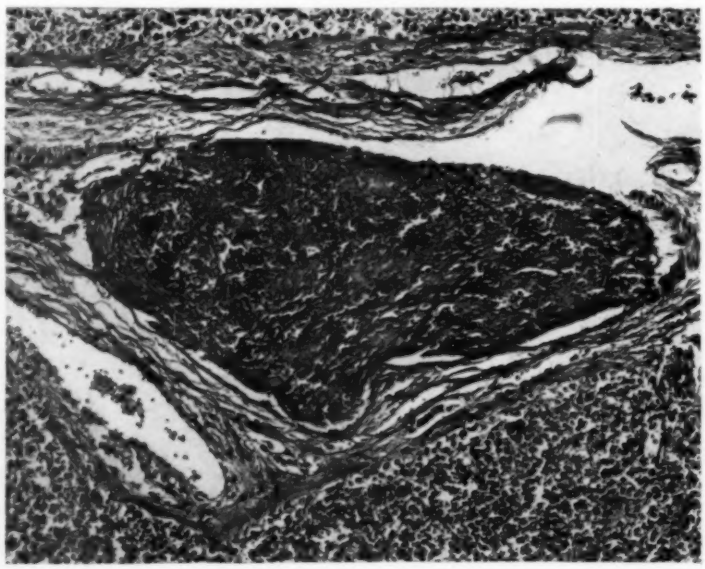

Fig. 5 (Case 4).—Relatively recent thrombus within an artery of the spleen. A recent infarct is present within the pulp supplied by this artery. Hematoxylin and eosin; reduced slightly from mag. × 110 (WRAH 14583 D).

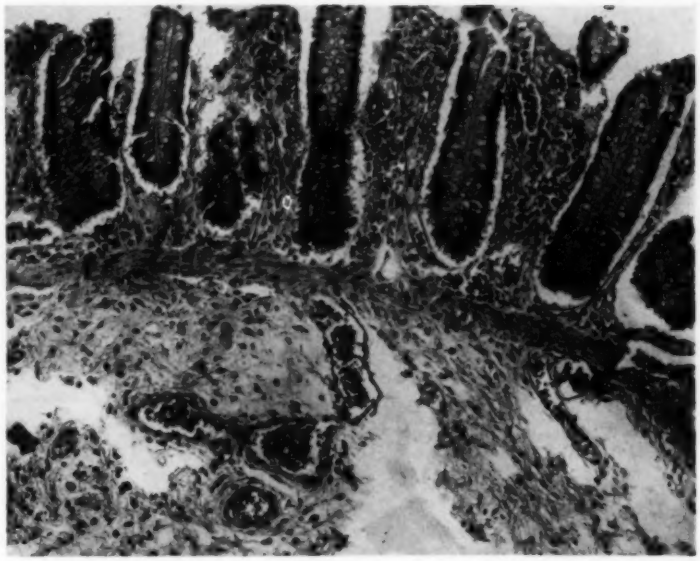

Fig. 6 (Case 4).—Section of intestine demonstrating numerous aggregates of polymorphonuclear granulocytes within the vessels of the submucosa. Chronic inflammation of the mucosal connective tissue and slight edema of the submucosa are other features. Hematoxylin and eosin; reduced slightly from mag. × 100 (WRAH 14583 G).

Kidneys. — Except for congestion, the gross appearance of the kidneys was not remarkable. The major microscopic alteration was the presence of glomerular capillary fibrin thrombi (Figs. 11 and 12). In some cases almost every glomerulus was involved, while in others only a few were thrombosed. The thrombi filled all the capillary loops in many glomeruli, while in others they were lodged in the precapillary arteriole or in only one or two loops of the tuft. Although infrequent, similar thrombi were found in the peritubular capillaries. Other changes consisted of colloid droplet "degeneration" of the proximal convoluted tubules adjacent to the glomeruli, hemoglobin casts, focal abscesses, and in two cases monilial abscesses.

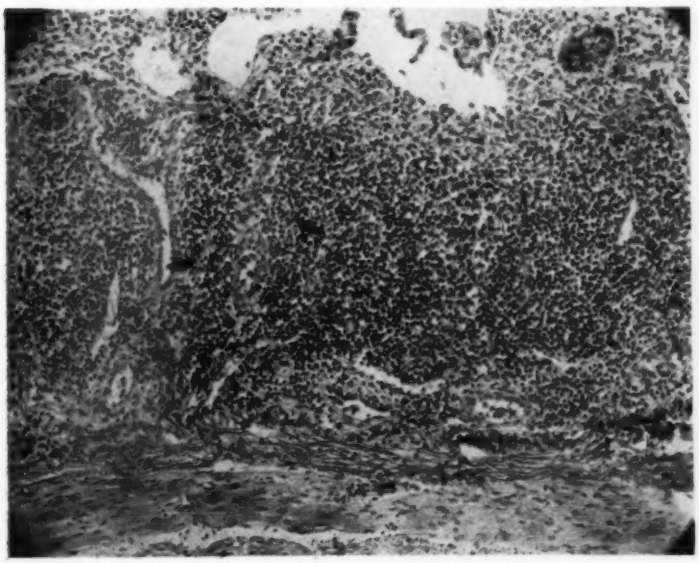

Fig. 7 (Case 6).—Severe chronic inflammation of the mucosa of the intestine is apparent in this section. The epithelial lining is completely destroyed. Hematoxylin and eosin; reduced slightly from mag. × 85 (WRAH 14436 G).

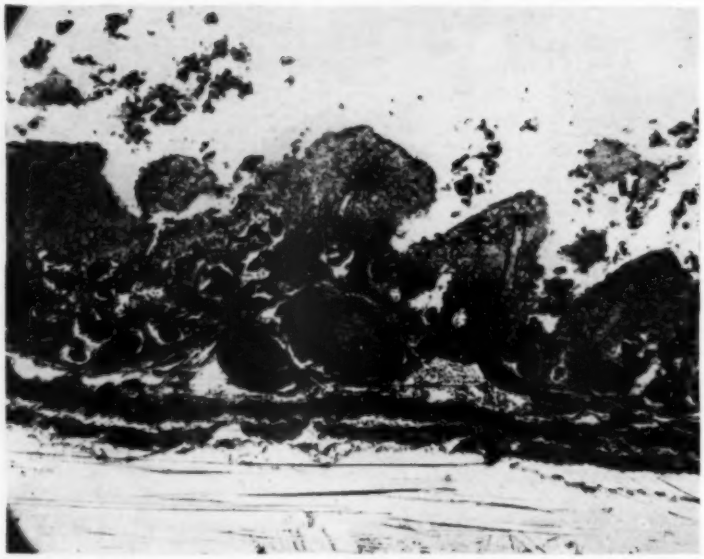

Fig. 8 (Case 4).—Section of the small bowel showing hemorrhagic necrosis of the mucosa and dilatation of the vessels of the mucosa and submucosa. Phosphotungstic acid hematoxylin; reduced slightly from mag. × 80 (WRAH 14583 C).

Brain.—Edema, vascular congestion, and subarachnoid, subdural, and intraventricular hemorrhages were noted grossly. Microscopically intracapillary fibrin thrombi were present in the majority of cases (Figs. 13 and 14). Some thrombi completely occluded the vascular lumen, while those in the larger vessels tended to coat the intima, leaving a central lumen. In three cases there was a perivascular leucocytic infiltrate, and in one case, necrosis of cerebral tissue (Fig. 15).

CLINICAL COURSE

The disease began as a mild process that progressively became severer. Initially mild injection of nasopharynx and vomiting were noted. Stools became loose, and then watery and profuse, with passage of mucus and blood terminally. Central nervous system symptoms developed in a few days and consisted of marked irritability, muscular hypertonicity, and occasionally convulsions with terminal coma. High temperature, abdominal

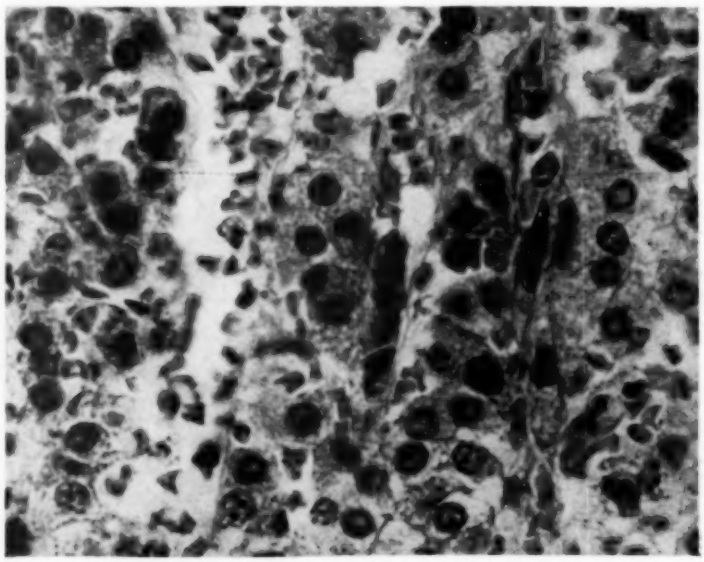

Fig. 9 (Case 4).—Adrenal cortex revealing a fibrin thrombus within a capillary of the zona fasciculata, near center of field. Hematoxylin and eosin; reduced slightly from mag. × 500 (WRAH 14583 J).

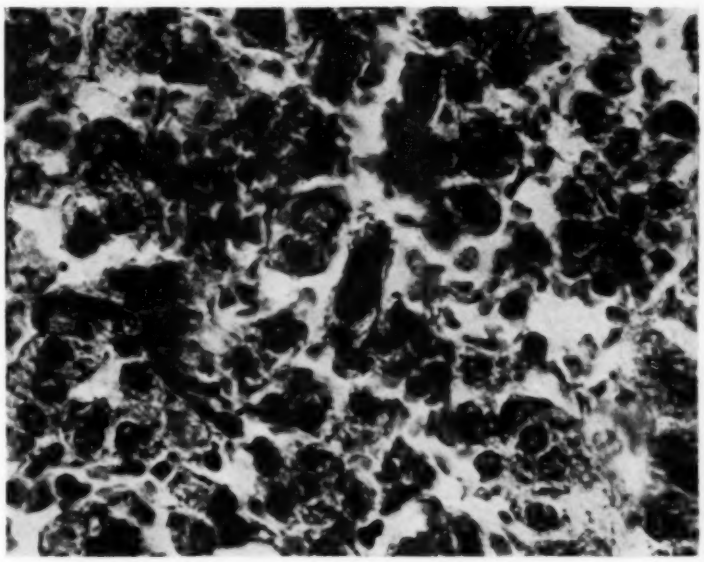

Fig. 10 (Case 4).—Adrenal cortex showing an intracapillary fibrin thrombus near the center of the field. Phosphotungstic acid hematoxylin; reduced slightly from mag. × 500 (WRAH 14583 B).

distention, dehydration, respiratory distress, and occasionally jaundice were noted.

Hypernatremia and hyperchloremia occurred in most patients, and these were corrected by fluid replacement therapy. In spite of this treatment periodic circulatory collapse occurred. Cortisone treatment during periods of circulatory collapse brought temporary improvement to a few infants, but in some was ineffectual. Nine of the infants died in circulatory collapse, with anuria, bloody stools, and coma.

A complete description of the epidemiology and clinical aspects of this epidemic has been reported by Belnap and O'Donnell.[18]

COMMENT

A. Clinical-Pathologic Correlation

The dehydration and acidosis in these infants appear to have been caused by the

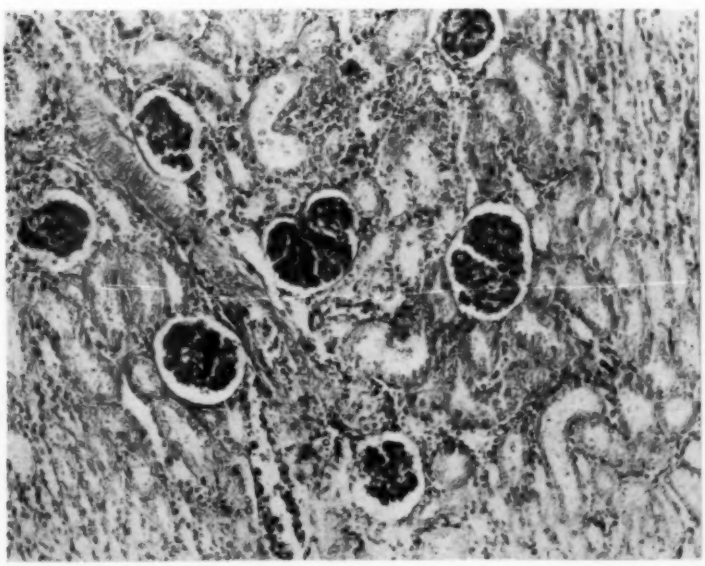

Fig. 11 (Case 6).—Kidney. Several glomeruli display intracapillary fibrin thrombi. Near the right lower corner of the photograph there is a fibrin thrombus within a peritubular capillary. Phosphotungstic acid hematoxylin; reduced slightly from mag. × 110 (WRAH 14436 E).

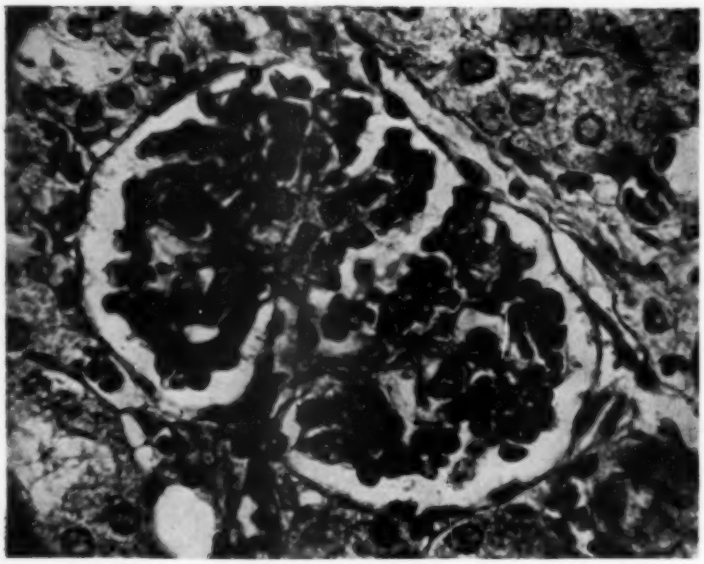

Fig. 12 (Case 6).—Kidney. Glomerular capillary fibrin thrombi. Hematoxylin and eosin; reduced slightly from mag. × 400 (WRAH 14436 I).

severe diarrhea, which in turn can be related to the diffuse chronic inflammation of the small and large bowel. The bloody stools originate from the areas of hemorrhagic necrosis of the small bowel and colon. The abdominal distention is due to paralytic ileus with dilated loops of small intestine. The marked edema of the connective tissue of the Auerbach plexi may indicate a direct toxic effect on these nerve cells as a possible cause of the intestinal dilation. Vomiting may be due to the diffuse inflammation and hemorrhage, which involve primarily the small intestine but which extend into the pyloric mucosa.

Shock in these cases is probably due to (1) dehydration, (2) relative adrenal insufficiency, and (3) direct effect of bacterial endotoxin. In the autopsied cases shock occurred in spite of adequate electrolyte and

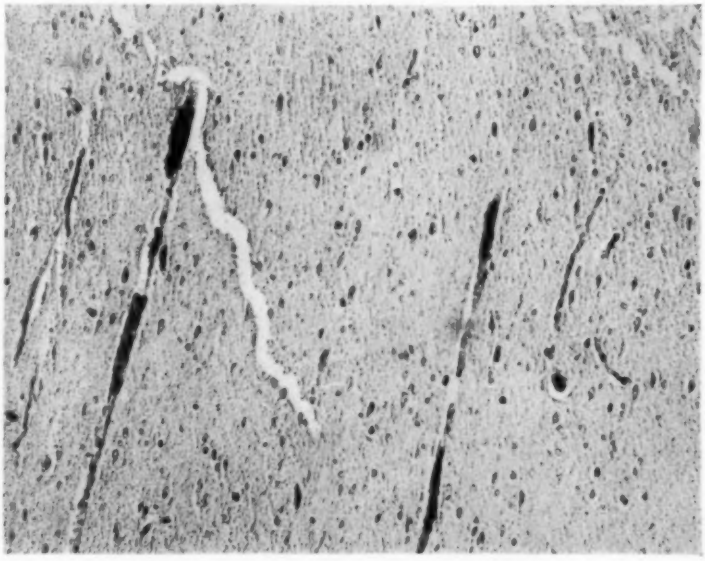

Fig. 13 (Case 1).—Section of brain revealing intracapillary fibrin thrombi. Phosphotungstic acid hematoxylin; reduced slightly from mag. × 110 (WRAH 14584 A).

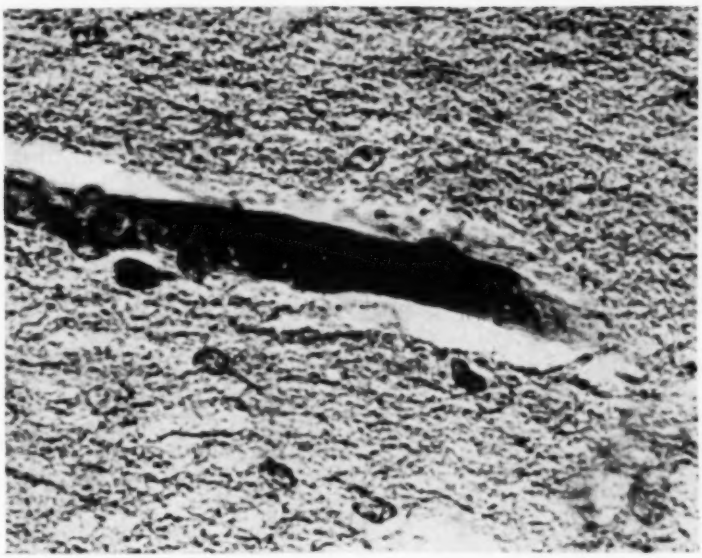

Fig. 14 (Case 1).—Section of brain revealing an intracapillary fibrin thrombus. Phosphotungstic acid hematoxylin; reduced slightly from mag. × 500 (WRAH 14584 B).

fluid replacement therapy and in spite of adrenocortical hormone replacement therapy. The possibility that this irreversible shock was caused by the disseminated intravascular coagulation must be considered. Schneider and Engstrom [19] have demonstrated that shock occurs in animals subjected to diffuse intravascular clotting. Shock is a common occurrence in other diseases in which diffuse intravascular coagulation occurs (McKay and co-workers [20]).

Anuria may be due to two causes: (*a*) dehydration and shock and (*b*) occlusion of glomerular capillaries by fibrin thrombi. The terminal anuria observed in these infants occurred in spite of the fact that the infants were well hydrated by fluid replacement therapy.

Jaundice in two of the cases may have been due to the presence of inspissated bile in the hepatic canaliculi.

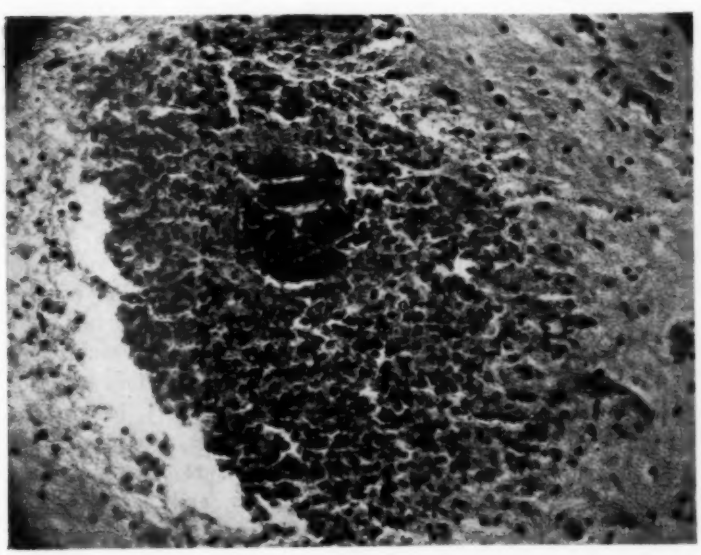

Fig. 15 (Case 3).—Section of brain demonstrating an intravascular occlusion of a small vessel with surrounding encephalomalacia and abscess formation. Phosphotungstic acid hematoxylin; reduced slightly from mag. × 200 (WRAH 14435 G).

Clinical evidence of adrenal insufficiency can be explained only on the basis of a relative adrenal insufficiency in these infants. There were no pathologic changes in the adrenals which would be adequate to explain this deficiency. The marked involution of adrenal tissue that occurs normally immediately after birth may be at the root of this problem.[21]

The most notable finding in this group is the fact that no infant survived the clinical combination of coma, shock, anuria, and the appearance of blood in the stools. These appear to be terminal events and offer a fatal prognosis.

B. Local and Generalized Shwartzman Phenomena in Epidemic Infantile Diarrhea

For the purpose of comparing the pathologic anatomy of infantile diarrhea with the Shwartzman phenomena, a brief review of the salient features of the latter will be presented.

The local reaction is produced in rabbits by an intradermal injection of bacteria or appropriate bacterial endotoxins. After 12 to 24 hours have elapsed, a mild local inflammatory reaction may be observed at the site of this injection. In control animals, this reaction gradually subsides during the next two or three days. If an intravenous injection of the same bacterial products is given, however, a severe reaction takes place at the site of the previous intradermal injection. This consists of the appearance of petechiae, which enlarge and coalesce to form a blue-black hemorrhagic necrotic lesion. This lesion reaches its greatest size in three to six hours following the intravenous injection. The intradermal injection is referred to as the "preparatory" injection, and the intravenous, as the "provocative" injection. The injections must be given in the order mentioned. That the reaction is nonspecific is evidenced by the fact that the bacterial products of one species of organism may be used to prepare the skin and those of another species, or even nonbacterial substances, may be used as provocative agents.[2]

In a histologic study of the pathogenesis of the local reaction, Stetson[2] found that the intradermal injection produced an interstitial inflammatory reaction with marked "perivascular cuffing" around capillaries and small veins. An hour after the intravenous injection the lumens of many of these vessels were occluded by masses of leucocytes and platelets. An hour or two later, necrosis of these vessels occurred. Four hours after the provocative injection, the vessels became greatly dilated and hemorrhage occurred into the connective tissue.

The generalized Shwartzman reaction is the systemic counterpart of the local reaction. It is produced by giving both the "preparatory" and the "provocative" injections intravenously. The gross lesions produced by such an experimental procedure consist of bilateral renal cortical necrosis and hemorrhage into the lungs, spleen, liver, lymph nodes, and gastrointestinal tract. The renal lesion is the key to the generalized reaction, and the key to the renal lesion is found in the glomeruli, which show the earliest change. This consists of an occlusion of the glomerular capillaries by fibrin masses. This lesion has been seen within a few hours after the second intravenous injection and occurs in all the kidneys that develop bilateral cortical necrosis.[22]

Most of the studies on the generalized reaction have been carried out by the use of bacteria-free toxins from various Gram-negative organisms, usually meningococci or typhoid or colon bacilli. Gratia and Linz[23] injected 5 cc. of B. coli-active filtrate intravenously into four rabbits. Two received 1 cc. of the filtrate intravenously 24 hours later. Two received four injections of 1 cc. of filtrate, diluted 1 : 1000, 1 : 100, and 1 : 10, intravenously. On the day following completion of the experiment, one of the rabbits of the first group and two of the second group died. Postmortem examinations showed blood in the peritoneal cavity, hemorrhage in the intestines, multiple petechiae in the kidneys, and hemorrhages into the lungs, lymph nodes, thymus, and bone marrow. Apitz,[24] in similar experiments using agar

washings of B. typhosus, produced similar results. The reaction in his experience was characterized by three main events: (*a*) death within 48 hours, (*b*) signs of a hemorrhagic diathesis, and (*c*) characteristic kidney lesions consisting of hemorrhage and necrosis.

The renal glomerular thrombi in the infants dying of epidemic infantile diarrhea are identical with those in the rabbits subjected to the generalized Shwartzman phenomenon. Zuelzer [25] has presented the evidence that bilateral cortical necrosis occurs in infants with diarrhea. Although present in the intestine rather than the skin, the areas of hemorrhagic necrosis in the gastrointestinal tracts of these infants are identical with those of the localized Shwartzman reaction. Such an interpretation points to the intestine as the portal of entry of the Shwartzman-active material in these infants. The fact that the toxins of various Gram-negative bacilli have long been known to produce this reaction, coupled with the fact that B. coli (of certain serotypes) have for some years been associated with outbreaks of epidemic diarrhea,‡ provides the evidence that this may be the toxin that is responsible for these tissue reactions.

The work of Sanarelli [33] some years before the recognition of the Shwartzman reaction as such seems particularly germane to the discussion of the pathogenesis of infantile diarrhea. Sanarelli infected rabbits with cholera bacilli and subsequently injected various Gram-negative bacterial toxins. The result of the injection was widespread hemorrhagic and necrotizing lesions in many tissues, including the kidney. The most striking lesions were observed in the wall of the gastrointestinal tract, where the bacilli had predominantly localized. Thomas [22] notes that

> The work suggests, in retrospect, that a systemic bacterial infection not only prepared the animal for the generalized Shwartzman reaction, but also produced an exaggeration of lesions in the tissues for which the microorganism had a particular affinity. Although the organism is different, the principles of bacterial localization and tissue response (hemorrhagic necrosis) appear to be the same in each of these conditions.

‡ References 26 through 32.

In summary, the probable pathogenesis of infantile diarrhea in this epidemic appears to have been initiated by an infection by a Gram-negative organism (E. coli, Serotype O111). The organism localized primarily in the small bowel and colon and produced a diffuse chronic inflammation of the intestinal mucosa. The endotoxin released by the organisms acted locally, thus "preparing" patches of the intestinal mucosa. Toxin was subsequently absorbed into the blood stream and acted as the "provoking" dose, with the consequent hemorrhagic necrosis of the localized Shwartzman reaction. In the course of time, a second "dose" of E. coli toxin was absorbed into the blood stream and resulted in the production of the generalized Shwartzman phenomenon, which appears to have been the basic cause of death in this group of infants. It should be noted at this point that single doses of certain bacterial toxins capable of eliciting the Shwartzman phenomena, if given in large enough amounts, may be rapidly lethal to animals without producing thrombi in any organs. Such a direct toxic effect may also have played some role in the deaths of these infants.

The demonstration of diffuse intravascular coagulation in these infants, whatever its pathogenesis may be, suggests the need for hematologic studies in the future. Episodes of intravascular clotting in other diseases of man and in experiments in animals are associated with certain fairly consistent alterations of the hemostatic mechanism. A sudden drop in the circulating platelets and leucocytes, a drop in the fibrinogen content, the development of fibrinolytic activity, and a slight prolongation of the prothrombin time are among the changes that have been observed in such conditions. Similar determinations in the victims of epidemic infantile diarrhea would supplement the histologic evidence of intravascular coagulation and would probably demonstrate the times in the course of the disease when the clotting episode or episodes occur.

SUMMARY

The pathological findings in nine infants who succumbed to an epidemic of infantile diarrhea due to Escherichia coli O111 have been described. Diffuse chronic inflammation of the intestinal tract, with foci of hemorrhagic necrosis in the mucosa of the small bowel and colon, and diffuse intravascular thrombosis of small vessels involving primarily the kidney, brain, spleen, and lung were the commonest pathological changes. The changes in the mucosa of the intestine are identical with the local Shwartzman reaction, while those in the kidney represent the generalized Shwartzman phenomenon.

The basic clinical manifestation of the Shwartzman reaction in these infants was coma or convulsion, shock, anuria or oliguria, and blood in the stools. This was the terminal episode in the majority of cases. It is suggested that studies of the hemostatic mechanism in infants with infantile diarrhea are indicated.

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News and Comment

GENERAL NEWS

Lecture by Dr. John G. Kidd.—Dr. John G. Kidd, Professor of Pathology, Cornell University Medical College, delivered the Sixth Augustus B. Wadsworth Lecture at the Division of Laboratories and Research, New York State Department of Health, Albany, N. Y., on Oct. 27. He spoke on "Immunological Approaches to the Problems of Cancer."

AEC Awards to Dr. John C. Bugher.—Dr. John C. Bugher, formerly of the Department of Pathology of the University of Michigan and retiring Director of the Division of Biology and Medicine of the U. S. Atomic Energy Commission, has received the AEC Distinguished Service Award and the Superior Accomplishment Award, the latter including a cash award of $2000.

Richard H. Jaffe Lecture by Dr. Esmond R. Long.—Dr. Esmond R. Long delivered the Richard H. Jaffe Lecture of the Institute of Medicine of Chicago on Nov. 14. This lecture was presented in a joint meeting of the Institute of Medicine, the Chicago Pathological Society, and the Society of Medical History of Chicago. The meeting, itself, was devoted to a memorial to the late Dr. Ludvig Hektoen.

Dr. S. E. Gould Retires as Editor-in-Chief.—Dr. S. E. Gould, editor-in-chief of the *American Journal of Clinical Pathology*, is resigning his position at the end of 1955. The editorial duties will be taken over by Dr. Parker R. Beamer, Professor of Pathology at Indiana University Medical School.

PERSONAL

Appointment of Dr. Joshua L. Edwards.—Dr. Joshua L. Edwards has been appointed professor and head of the Department of Pathology at the University of Florida College of Medicine, Gainesville, Fla. Since September, 1953, Dr. Edwards has been assistant in the Department of Pathology and Microbiology in the Rockefeller Institute for Medical Research in New York City.

Books

The Biologic Effects of Tobacco, with Emphasis on the Clinical and Experimental Aspects. Edited by Ernest L. Wynder, M.D. Price, $4.50. Pp. 215, with 20 illustrations. Little, Brown & Company, 34 Beacon St., Boston, 1955.

This small book contains seven chapters by various authors on the following aspects of tobacco and its effects: chemistry, by Kosak; pharmacology, by Kensler; cardiovascular system, by McDevitt and Wright; neoplastic diseases, by Wynder; gastrointestinal tract, by Batterman; allergy, by Lowell; cause and effect, by Hammond. These chapters, except for the last, are essentially brief reviews containing little that is new and varying in comprehensiveness and in critical quality. Most of them are good and free of emotion. The remarkable fact emerges from a large amount of observation and investigation, as pointed out by Garland in a foreword, that tobacco usage has remarkably few obvious physiological and pathological effects compared with alcohol, which may cause such striking conditions as drunkenness, delirium tremens, and death. Although this volume leaves the subjects about where it found them, it serves a useful purpose by collecting much information on a timely subject.

A Textbook of Physiology. Edited by John F. Fulton, M.D. Price, $13.50. Pp. 1275, with 600 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1955.

This is the seventeenth edition of a widely used textbook of physiology especially known for its section on neurophysiology. The first seven chapters covering the principles of nervous activity have been reorganized by the same author as in the last edition. A few new illustrations and recent references have been added. A new chapter on acetylcholine and energy transformations in nerve cells presents important recent developments. The previous chapter on the hypothalamus has been revised, with the addition of a new chapter on the limbic system.

The section on the properties and constituents of the blood has been brought up to date, with an extensive reworking of the chapter on coagulation of blood. From the many conflicting theories and recent developments in the study of the coagulation of blood, an integrated and logically developed point of view is presented.

In the section concerned with the neurogenesis of respiration, evidence from recent investigations is presented against the view that the respiratory center exists as a well-compartmented mass of nerve cells. The entire chapter is rewritten in a refreshing manner and is integrated with the following chapters on the regulation of respiration.

The chapter on kidney function has been completely rewritten and expanded by a new author. It reflects the change in emphasis of renal studies over the past five years and incorporates recent findings on the tubular mechanisms. Consideration is given to neurohormonal regulating mechanisms.

The general structure and organization of the book remains unchanged, but it is slightly longer. This seventeenth edition continues to offer current fundamental information for both the medical student and the physician.

Year Book of Pathology and Clinical Pathology (1954-1955 Series). Edited by William B. Wartman, M.D. Price, $6.00. Pp. 486, with 168 illustrations. The Year Book Publishers, Inc., 200 East Illinois St., Chicago 11, 1955.

This book, the second one under the editorship of Dr. William B. Wartman, contains an introductory article by him on The Scope of Pathology; a special article by James W. Riegan on Cancer in Situ; one by Samuel P. Hicks on Experimentally Induced Developmental Abnormalities of the Central Nervous System; one by Israel Davidsohn on The Anemias, and one by William S. Hoffman on Serum Sodium Concentration and Its Therapeutic Significance to the Clinical Pathologist. In addition, the usual brief series of selected articles from current literature are presented with editorial comments here and there. The book is, as usual, well printed and well indexed, both as to authors and subject, and offers a current source of reference in many recent articles in the field of general and clinical pathology.

Pathology. By Peter A. Herbut, M.D. Price, $16.00. Pp. 1227, with 1378 illustrations on 651 figures. Lea & Febiger, Washington Square, Philadelphia 6, 1955.

This book is an encyclopedic compilation presented in standard textbook size. A terse style and rather rigid pattern is used. Each disease is considered in the following sequence: definition, incidence, cause, gross appearance, microscopic appearance, complications, clinicopathologic correlation, diagnosis, treatment, and prognosis. The pathologic anatomy is given much emphasis. The material is based upon the author's didactic lectures in pathology given to medical students.

The first one hundred fifty pages contain the history of pathology, discussion and instructions for the autopsy, and a section on general pathology. The discussions of the basic pathologic processes are divided into chapters on congenital anomalies, degenerations, inflammations, physical disturbances, and tumors.

This division is carried through the remaining chapters. Each chapter includes consideration of an organ or part of an organ system. The organs are roughly grouped by systems except for the endocrines, which are taken up in sequence with anatomically related organs. This organization plus the adherence to the outlined general plan makes it difficult to get an integrated picture of a disease affecting multiple organs or systems. This difficulty is encountered with the consideration of diseases of the reticuloendothelial system, blood, spleen, and lymph nodes in separate chapters. The adherence to a system of this type seems to interfere with proper emphasis of important material.

The text is amply illustrated; the black and white photographs are well chosen and executed. The color plates are few in number and are not uniformly of the same quality.

This book represents a large and up-to-date addition to the currently available textbooks of pathology. The basic problem of how best to organize a textbook remains controversial, but this represents a good example of wide coverage of material, systematically but briefly treated. With the constantly expanding mass of material, it may soon be necessary to stress basic principles in general textbooks and consider systematic organ or tissue disease in separate monographs.

Sharpey-Schafer's Essentials of Histology. By H. M. Carleton and R. H. D. Short. Price, $6.50. Pp. 661, with 659 figures. Longmans, Green & Co., Inc., 55 Fifth Ave., New York 3, 1954.

This volume is the sixteenth edition of a well-known English textbook of histology. The previous edition was published in 1949. The revision includes replacement of several pages of blood cells by color plates, some change in the text, and the separation of the technical instructions into an appendix. The general character of the book has remained unchanged. No extensive effort has been made to present the chemical composition of substances where recent work has contributed significantly to the understanding and nature of cells and tissues. As the name implies, this book contains only the essentials of the subject. There is emphasis on description and definitive anatomy. Genetic and embryologic considerations are briefly reviewed.

This book is recommended for general courses in biology and for use by medical students as a clearly written source for review.

The Human Adrenal Cortex (Ciba Foundation Colloquia on Endocrinology). Edited by G. E. W. Wolstenholme and Margaret P. Cameron. Price, $10.00. Vol. 8. Pp. 665. Little, Brown & Company, 34 Beacon St., Boston 6, 1955.

This volume contains the proceedings of the Conference on the Human Adrenal Cortex held in April of 1954. Participating in the conference were many of the outstanding students in this general field. The meeting was held under the joint chairmanship of Dr. Gregory Pincus and Dr. R. W. Thorn. The presentations together with the discussions, are included, and there has been a minimum of editing. Students of the adrenal gland will find in this volume discussions of most, if not all, of the current problems dealing with the adrenal gland.

Quantitative Methods in Histology and Microscopic Histochemistry. Olavi Eranko. Price, $5.50. Pp. 160, with 49 illustrations. Little, Brown & Company, 34 Beacon St., Boston 6, 1955.

It is a great pleasure to see the appearance of a book of this title and content in the rapidly growing field of histochemistry. Although we are still in the beginning of the era of quantitation in histology and histochemistry, its importance cannot be underestimated. This small volume contains in essence an introduction to quantitative methods in experimental biology. There is

an elementary introduction to the problem of sampling and the measures of variability. Some notes on setting up of animal experiments including the principles of randomization and size of animal groups are given. The remaining material deals with the problem of quantitation of tissue cells, parts of the cells, and chemically demonstrable susbtances. The techniques described for these measurements are those which, for the most part, use simple equipment. In each instance the principles of the method are emphasized and the limitations are pointed out. Techniques are not described in great detail, but references are given indicating where these may be found. A final chapter deals with the more complicated statistical analysis of the quantitative measurement not covered in the introductory chapters to the book. Within the confines of this small volume is contained the essence of some conceptions which have been developing steadily and are of fundamental importance to the furtherance of modern experimental biology and pathology. It will be well worth the while of any modern microscopist, and particularly those interested in investigation in this field, to have this small book available.

Hypophyseal Growth Hormone, Nature and Actions. Editors, Richmond W. Smith, Jr., Oliver H. Gaebler, and C. N. H. Long. Price, $12.00. Pp. 576. McGraw-Hill Book Company, Inc., 330 W. 42nd St., New York 36, 1955.

This book records the proceedings of the international symposium, The Hypophyseal Growth Hormone, Nature and Actions, which was held at the Henry Ford Hospital, in Detroit, in 1954. Participating in this symposium were many of the outstanding students in this field. Their papers and the discussions are included, so that all students of endocrinology have available in this book the material considered at the symposium. There are many references to recent literature, so that this volume should be of great value, particularly to endocrinologists.

Collected Papers of the Mayo Clinic and the Mayo Foundation. Edited by R. M. Hewitt, and others. Price, $12.50. Vol. 46. Pp. 843, with 189 illustrations. W. B. Saunders Company, 218 W. Washington Sq., Philadelphia 5, 1954.

This volume, the forty-sixth in the series of publications from the Mayo Clinic and the Mayo Foundation, consists of six hundred twenty-nine papers covering the period between December, 1953, and November, 1954. The material was selected primarily with "the interests of the general practitioner, the general surgeon, and the diagnostician in mind." The articles are grouped by systems, and there is both an index of contributors and an index of subjects. It is, therefore, easy to ascertain the current thinking of members of the Mayo Clinic on the many problems discussed.

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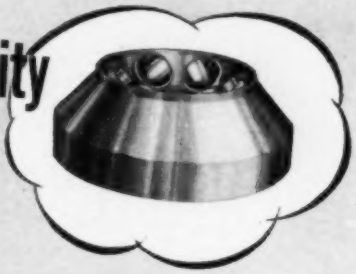

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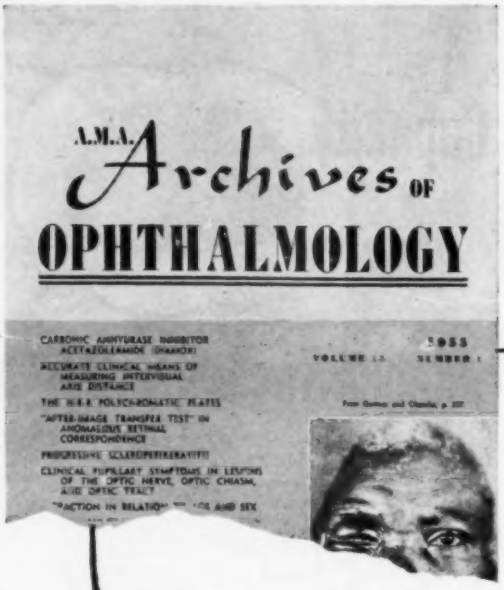
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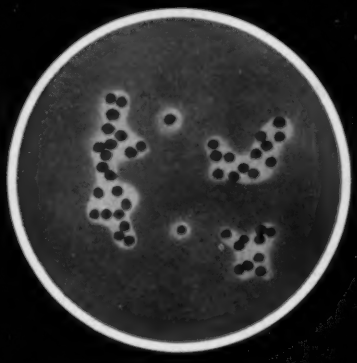

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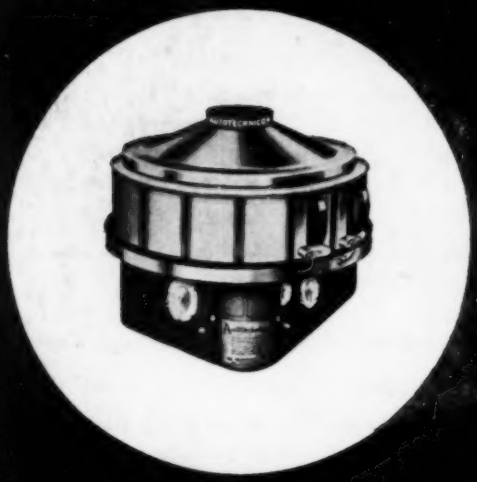

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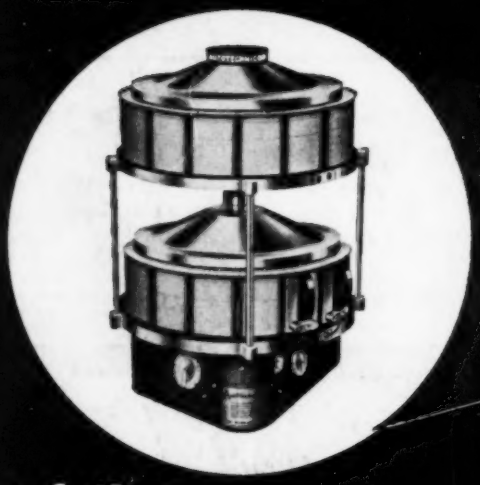

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